

NOTE

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**INVESTIGATION OF THE ROLE OF THIOREDOXIN IN THE INVASIVE
PHENOTYPE AND ITS INTERACTION WITH THE TRANSCRIPTION
FACTOR Sp1**

by

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ABSTRACT

Thioredoxin is a small ubiquitous oxido-reductase found in all species. The highly conserved active site, which facilitates thioredoxins redox activity, contains two redox active cysteine residues. Thioredoxin has numerous protein substrates to which it donates H⁺ ions and it can also function as a free radical scavenger. Through these activities thioredoxin is able to influence the redox state of not only its protein targets, but also the entire cellular environment.

Thioredoxin has been implicated in many biological functions, and one mechanism by which it influences these functions is through interactions with a number of transcription factors including NF- κ B and p53. Thioredoxin also has numerous extracellular biological roles. It has been shown that thioredoxin is actively secreted from a number of normal and transformed cell lines including fibroblasts and activated B and T cells

This study investigates the role of thioredoxin in embryonic implantation and cancer cell metastasis, two physiological functions which rely on the same basic processes. Thioredoxin expression has previously been shown to be increased in many cancers. However it has not yet been established whether this increase is a causative or a side effect of the cancerous phenotype. Similarly thioredoxin expression has previously been shown to be increased during different phases of the oestrus cycle and pregnancy.

This thesis describes the role of thioredoxin in embryonic implantation using a marmoset model. A thioredoxin cDNA was isolated and subsequently sequenced. Preliminary antibody experiments indicated that the anti human thioredoxin monoclonal antibodies available in our laboratory would recognise marmoset thioredoxin. Subsequently immunocytochemistry using anti human thioredoxin antibodies was carried out on sectioned marmoset uterus and embryonic tissue. The results indicated that thioredoxin is expressed by cells at the embryonic-maternal interface of early implantation sites. Further studies demonstrated that thioredoxin is also expressed and secreted by cultured blastocysts *in vitro*.

This thesis also describes the role of thioredoxin in cancer cell metastasis. Results of this study indicate that thioredoxin is actively involved in facilitating the

invasive phenotype of breast cancer cells. The two cell lines utilised were MCF-7, a well differentiated, relatively non-invasive breast cancer cell line; and MDA-MB-231, a poorly differentiated, highly invasive breast cancer cell line. The cell lines were transfected with thioredoxin sense, antisense and 1SS (encodes thioredoxin with both active cysteine residues mutated to serine residues and is thus redox inactive) constructs.

The results demonstrate that when endogenous thioredoxin levels are increased, i.e. transfected with a sense thioredoxin construct, the invasive breast cancer cell line MDA-MB-231 becomes more invasive, conversely when endogenous levels are decreased, i.e. transfected with antisense or 1SS constructs, the invasive capacity of these cells decreases. However, when the endogenous level of thioredoxin was manipulated in the relatively non-invasive cell line MCF-7 very little effect was observed. Results also indicate that thioredoxin has the ability to act as a chemoattractant for actively invading breast cancer cells. Both of these functions appear to be dependent on thioredoxin's redox activity.

Additional studies described in this thesis have shown that thioredoxin is involved in the regulation of Sp1 *in vitro*. Sp1 is a transcription factor known to regulate the transcription of a number of genes whose products are intimately involved in the invasive phenotype. The results in this study suggest that Sp1 DNA binding is regulated by thioredoxin such that when reduced by the enzyme its binding to DNA is facilitated. Results also indicate that Sp1 may regulate the transcription of thioredoxin by binding to Sp1 sites within the thioredoxin promoter.

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STATEMENT OF ORIGINALITY

This work has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.

K. L. Bloomfield

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LIST OF ABBREVIATIONS

ARE: Antioxidant Response Element	mRNA: messenger RNA
bp: base pairs	OD: Optical Density
BSA: Bovine Serum Albumin	O/N: Overnight
cDNA: copy DNA	ORE: Oxidative Stress Response Element
DEM: Diethyl Malate	
DMF: N,N-Dimethylformamide	PAGE: Poly-Acrylamide Gel Electrophoresis
DMSO: Dimethyl sulfoxide	
DNA: Deoxyribonucleic Acid	PBS: Phosphate Buffered Saline
dNTP: deoxynucleotide triphosphate	PCR: Polymerase Chain Reaction
DTT: Dithiothreitol	PEG: Polyethylene Glycol
E.coli: Escherichia coli	PNK: Polynucleotide Kinase
ECM : Extracellular Matrix	RNA: Ribonucleic Acid
EDTA: Ethylenediaminetetra Acetic Acid	ROS: Reactive Oxygen Species
	rpm: revolutions per minute
EMSA: Electromobility Shift Assay	SCM: Serum Containing Media
ERE: Estrogen Response Element	SDS: Sodium Dodecyl Sulfate
EPF: Early Pregnancy Factor	TEMED: N,N,N',N' – Tetramethylethylenediamine
FCS: Fetal Calf Serum	
FSH: Follicle Stimulating Hormone	TIMP: Tissue Inhibitor of Metalloproteinase
hCG: Human Chorionic Gonadotrophin	
HIV: Human Immune Deficiency Virus	TRed: Thioredoxin Reductase
HRP: Horse Radish Peroxidase	Trx: Thioredoxin
ICM: Inner Cell Mass	TNF: Tumour Necrosis Factor
IPTG: Isopropyl- β -D-thiogalactopyranoside	UTR: Untranslated Region
	UV: Ultra Violet
LB: Luria Broth	
LH: Leutinising Hormone	
MMP: Matrix Metalloproteinase	

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