THE ROLES OF SECRETED PROTEIN ACIDIC AND RICH IN CYSTIENE (SPARC) IN INTESTINAL INFLAMMATION, HEALING AND FIBROSIS.

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BSc(Hons.)

This thesis is presented for the degree of Doctor of Philosophy of Murdoch University,

School of Veterinary and Biomedical Sciences,

2012

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

.....
(Yoke-Leng NG)

ABSTRACT

Secreted Protein Acidic and Rich in Cysteine (SPARC) is a matricellular protein expressed during tissue repair and regulates cell proliferation and migration. It binds to, and interacts with collagen and regulates matrix metalloproteinase (MMP) expression. The aim was to determine if SPARC modifies intestinal inflammation, healing and fibrogenesis.

Intestinal disease was investigated using SPARC null (KO) and wild-type (WT) mice in which inflammation was induced by 3% dextran sodium sulphate (DSS) in the drinking water for 7 days. Inflammation was assessed endoscopically, clinically and histologically on days 7, 14, 21 and 35 after initiation of DSS treatment. Systemic and colonic cytokines and chemokines were quantitated by ELISA and CBA. Colon, mesenteric lymph node and spleen were analysed by flow cytometry and immunofluorescence for inflammatory cell infiltrates. To determine the effect of SPARC on the extracellular matrix (ECM) genes regulation, RNA from colonic tissue, and colonic myofibroblasts from WT and KO mice, were analysed by real time PCR for expression of ECM related genes.

KO animals had significantly lower endoscopic scores of inflammation, suffered less weight loss, diarrhoea, faecal blood and had lower spleen/body weight ratios compared to WT animals consistent with less colonic and systemic inflammation. WT mice had higher levels of histological inflammation and damage when compared to KO animals and in the majority of KO animals the colonic mucosa had completely regenerated by day 35 in contrast to the WT mice. Compared to WT mice, in KO animal colons at day 7 there was significantly less IL1β and MIG expression while TGFβ1 levels were higher. Flow cytometric analysis identified a significantly greater percentage of FoxP3+ regulatory T cells in the spleen and draining lymph nodes of KO animals. KO mice also

had fewer of cells, such as CD68+ macrophages and Ly6G+ neutrophils, of the innate immune e system infiltrating the inflamed colon.

Collagen (Col) 1α1, Col3α1 MMP13 and MMP3 expression levels were reduced in DSS-treated WT colons at day 7 and these were significantly lower than those observed in the KO colons. TIMP1 expression was significantly lower in KO mice at day 35 when healing was complete in this group. TIMP2 and TGFβ1, TGFβ3 were not different between the groups at any time point. The observation by others that collagen fiber diameters in KO colons were noted to be significant smaller than in WT animals suggesting that SPARC modifies the collagen bundling. Compared to unstimulated WT fibroblasts, KO cells had lower Col1α1 and Col3α1 expression. Stimulation with PMA reduced Col1α1 and Col3α1 and increased MMP13 and TIMP1 expression in all the isolated cells, but PMA had no effect on MMP3, TIMP2, TGFβ1 and TGFβ3 expression.

DSS induced less colonic and systemic inflammation in KO compared to WT mice and the inflammation appeared to resolve faster. This may be secondary to increased numbers of regulatory T cells and increased colonic TGF-β levels which may inhibit effector cell activity, including cytokine and chemokine expression and aid in the more rapid resolution of inflammation and restoration of the intestinal mucosa. SPARC is able to modify tissue healing potentially through the regulation of collagen expression, bundling and its degradation by MMPs, which impacts on tissue turnover rate and thus delays healing. Hence, SPARC might represent a promising therapeutic target in clinical management of inflammatory bowel diseases (IBD).

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ACKNOWLEDGEMENTS

First of all, I wish to express my deepest gratitude to my supervisor Prof. Ian Lawrance for giving me the opportunity to extend my study in his lab, as well as his continuous guidance and support throughout the course of my PhD. My sincere appreciations also go to my co-supervisors: To Dr Borut Klopcic for his encouragement, expertise and critical yet helpful comments on my research project and thesis; and to Dr. Wayne Greene for his valuable comments and suggestions regarding my project and administrative matters.

I'd especially like to thank A/Prof Jane Allan for proofreading my thesis and being patient with me on lab related issues. A great "Terima Kasih" to Dr. Anita Chua for her expert guidance and friendship throughout. You two was like my extra "uncredited" supervisors who always willing to help and support me academically and emotionally.

I was really lucky to work in Freo's IBD research group because of the following great people. Mrs. Frances Lloyd, the super RA, who provided technical brilliance to me right from my honours till now and all those delicious sweet treats. Constant moral support and guidance was also given to me by Dr. Angela Chew, who always broadened my thinking and boast up my caffeine levels. My fellow PhD student, Kristine Fu, thanks for cutting my cyrosections and for your histo knowledge, not to mention that all the fun and jokes you brought to me (your mum's home cooking too). Thanks girls.

Sincere thanks must go to those that helped in term of technical support throughout my PhD. Alison and Carla from the animal facility at Fremantle Hospital for their invaluable assistance. Staff from the Pathwest Histopathology Department at Fremantle Hospital, in particular; Dr Cynthia Forrest for her knowledge and precious time on the histological scoring. Staff from the Centre for Microscopy, Characterisation and Analysis, UWA, helped me regarding flow cytometry, immunofluorescence and TEM, especially Lyn Kirilak; Dr Leanne Scott from the statistics department, Murdoch

University, for her statistical advice, HDR scholarships officer, Julie Blake from the Murdoch University for her assistance with my scholarship extension. Many thanks to Irma Larma for her tireless technical support with the flow cytometry; both present and past lab members at MSL, Fremantle Hospital: Carly, Amber, Gwen, Ross, Ro, Desiree, Brenda, Kerry, Janina and many more for their continual assistance throughout my work.

To family and friends, thanks for providing me with unconditional love and support in every way. Last but very essential, I truly appreciated the Murdoch University Research Scholarship that providing me the financial support.

I thank you all again (those that have been mentioned and sorry for those that I missed) from the bottom of my heart. I couldn't have done it without your support.

SPECIAL DEDICATION

To my parent, thanks for all the hard work and sacrifices you have made to allow me to pursue my dream and choose the path I want; forgive me for being "irresponsible" child sometimes. Nothing can express my thankfulness and how grateful I am besides dedicated this to you both.

&

The "MouthSky Gang" and Aunty K. Lan, thanks for putting up with me all the time, providing me with support, encouragement and most importantly "babies's stories" to brighten these tough years.

CONFERENCE ABSTRACTS

Ng Y.L., Klopcic B., Lloyd F, Greene W, Lawrance IC "Combined Transforming Growth Factor-β1 and -3 Fibroblast stimulation modifies the Extracellular Matrix gene expression through Smad independant signalling"
Poster presented at Digestive Diseases Week – May 2007; Washington, DC
Gastroenterology Volume 132, Issue 4, Supplement 1 A568 (April 2007) Annual Abstract Supplement

Ng Y.L., Klopcic B., Greene W, Lawrance IC "Combined Transforming Growth Factor-β1 and -3 Fibroblast stimulation modifies the Extracellular Matrix gene expression through Smad independant signalling"
Oral presented at Australian Society for Medical Research (ASMR)- June 2007; Perth, Western Australia

Ng Y.L., Klopcic B., Lloyd F, Greene W, Lawrance IC "SPARC attenuated Intestinal Inflammation in the DSS-treated Mouse?"
Poster presented at 9th Euroconference on Clinical Cell Analysis- September 2009; St. Etienne, France

Ng Y.L., Klopcic B., Lloyd F, Greene W, Lawrance IC "A role for SPARC in modifying inflammation and healing in the DSS murine model of colitis"
Poster presented at 18th united European Gastroenterology Week- October 2010; Barcelona, Spain

Ng Y.L., Klopcic B., Lloyd F, Fu S.K., Lawrance I.C. "SPARC modifies colonic tissue healing and inflammation by regulating collagen and MMP expression"
Oral presented at 7th Congress of European Crohn's and Colitis Organisation – February 2012; Barcelona, Spain

Ng Y.L., Klopcic B., Lloyd F, Greene W., Lawrance I.C. "SPARC enhances intestinal inflammation in dextran sodium sulphate-induced murine colitis"
Poster presented at 7th Congress of European Crohn's and Colitis Organisation – February 2012; Barcelona, Spain

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ABBREVATIONS

The following abbreviations are used throughout this thesis:

α-SMA	α-smooth muscle actin
AF	Alexa fluor®
APC	Antigen presenting cells
BCP	Bromochloropropane
BSA	Bovine serum albumin
CCL28	Mucosal epithelial chemokine
CD	Cluster of differentiation
cDNA	Complementary deoxyribonucleic acid
COX	Cyclooxygenase
CrD	Crohn's disease
CTGF	Connective tissue growth factor
DC	Dendritic cell
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulphoxide
DSS	Dextran sodium sulphate
DTT	Dithiothreitol
ECM	Extracellular matrix
EDTA	Ethylene diamine tetra acetic acid
FBS	Foetal bovine serum
FSC	Forward scatter
FITC	Fluorescein isothiocyanate
FoxP3	Forkhead box P3
GALT	Gut-associated lymphoid tissue
GIT	Gastrointestinal tract
H&E	Haemotoxylin and eosin
HBSS	Hank's balanced salt solution
HCl	Hydrogen chloride
HEPES	N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid
IBD	Inflammatory bowel diseases
IEL	Intraepithelial lymphocytes
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
ILF	Isolated lymphoid follicles
KCl	Potassium chloride

$KHCO_3$	Potassium hydrogen carbonate
KO	Knockout
LAP	latency-associated peptide
LP	Lamina propria
LPMC	Lamina popria mononuclear cell
LPS	lipopolysaccharide
LTBP	Latent TGF-β binding protein
MAdCAM	Mucosal vasacular addressin cellular adhesion molecules
MgCl	Magnesium chloride
M cell	Specialised epithelial cells in GLT
MCP-1	monocyte chemotactic protein-1, CCL2
MEICS	Murine endoscopic score of colitis severity
MHC	Major histocompatibility complex
MIG	Monokines induced by IFN-γ, CXCL9
MIP	Macrophage inflammatory proteins
MLN	Mesenteric lymph node
MMP	Matrix metalloproteinase
Na_2CO_3	Sodium carbonate
NaCl	Sodium chloride
NaN_3	Sodium azide
NaOH	Sodium hydroxide
NF	Nuclear factor
NH_4Cl	Ammonium Chloride
NOD	Nucleotide oligomerisation domain
OD	Optical density
Oligo	Oligonucleotide
PAI-1	Type-1 plasminogen activator inhibitor
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PE	Phycoerythrin
PRR	Pattern recognition receptors
PMA	Phorbol 12-myristate 13-acetate
PMN	polymorphonuclear leucocytes
PDGF	platelet derived growth factor
PG	Prostaglandin
PP	Peyer's Patches
PSF	Penicillin /Streptomycin/Fungizone

RANTES	Regulated on activation normal T cell expressed and excreted, CCL5
RNAse	Ribonuclease
RNAsin	Ribonuclease inhibitor
ROR	Retinoic-acid-receptor-related orphan receptors
RT	Room temperature
SEM	Standard error of mean
SPARC	Secreted Protein Acidic and Rich in Cystiene
SSC	Side scatter
STAT	Signal transducer and activators of transcription
TBS	Tris-buffered saline
TBST	TBS-Tween
TGF	Transforming growth factor
Th cell	T helper cell
TIMP	Tissue inhibitors of metalloproteinases
TLR	Toll-like receptors
TNBS	trinitrobenzenesulfonic acid
TNF	Tumour necrosis factor
tPA	tissue type plasminogen activator
Treg cell	Regulatory T cell
TREM-1	Triggering Receptor Expressed on Myeloid cells-1
UC	Ulcerative colitis
uPA	Urokinase type plasminogen activator
UV	Ultra-violet
VEGF	Vascular endothelial growth factor
WT	Wild type