The Biological Role of Extracellular Matrix in Ovarian Cancer Metastasis.

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SUMMARY

Ovarian cancer metastasis is characterized by the shedding of malignant cells from the surface of the ovary and their implantation onto the peritoneal surface which lines the abdominal cavity. As the factors promoting this process are poorly understood, we investigated the ovarian cancer–peritoneal interaction by means of *in vitro* co-culture experiments with ovarian cancer (OVCAR-3, OVCAR-5, and SKOV-3) and peritoneal (LP-9) cells. In this system, we identified by mass spectrometry that levels of transforming growth factor β inducible protein (TGFBIp), periostin, fibronectin, plasminogen activator inhibitor-1, cytokeratins 1, 5, 6C, 9, 10, 14, and 16, transketolase, annexin A2, annexin A6, and elongation factor-2 were modulated as a result of direct contact between peritoneal and ovarian cancer cells or through interactions via shared media.

We went on to investigate the functional role of the extracellular matrix (ECM) protein, TGFBIp in ovarian cancer. Immunohistochemistry showed high TGFBIp levels in normal surface ovarian epithelial and peritoneal cells whilst in comparison, TGFBIp levels in primary serous ovarian carcinomas and matching metastatic implants were greatly reduced. In functional *in vitro* experiments, rTGFBIp significantly increased the motility and invasion of OVCAR-5 and SKOV-3 cells and significantly increased ovarian cancer cell (OVCAR-5, OVCAR-3 and SKOV-3) adhesion to peritoneal (LP-9) cells which was reversed by addition of a neutralizing TGFBIp antibody. We also demonstrated that the increases in OVCAR-5 cell adhesion, motility, and invasion, were independent of the Arg-Gly-Asp (RGD) motif in the C-terminal domain of TGFBIp. We conclude that TGFBIp expressed by peritoneal cells increases the metastatic potential of ovarian cancer cells. TGFBIp is therefore a potential novel therapeutic target against ovarian cancer.

Further investigation determined that secreted TGFBIp was processed at both the N- and C-terminal domains during ovarian cancer–peritoneal cell co-culture in the same amino acid range as that of TGFBIp cleaved by plasmin. Plasmin was found to be upregulated within 1 hr of co-culture and TGFBIp processing in the *in vitro* co-culture system could be blocked by a plasmin inhibitor, 6-aminocaproic acid (ε-ACA) and a broad spectrum protease inhibitor which inhibits plasmin but not matrix metalloproteinases (MMPs). Furthermore, the processing was not blocked by an MMP inhibitor, GM6001. We therefore conclude that TGFBIp is cleaved by plasmin and not an MMP during peritoneal-ovarian cancer co-culture.

In summary, these studies have shown, that when peritoneal cells are allowed to interact with ovarian cancer cells, whether by direct contact or by shared growth media which occurs at different steps of ovarian cancer metastasis, a proteolytic response is triggered.

We also investigated the expression of other ECM components in ovarian cancer; the proteoglycan versican, the polysaccharide hyaluronan (HA), and one of its receptors, CD44, in ovarian cancer tissues and their role in the metastatic behaviour of ovarian cancer cells. We found that a higher proportion of serous ovarian carcinoma had high stromal versican when compared with normal ovary and high stromal CD44 when compared with normal and benign serous tumours. Although high stromal versican was positively correlated with high stromal HA, stromal HA was not increased in serous ovarian carcinoma when compared with normal ovary or benign serous tumours.

We determined that the assembly of a HA-versican pericellular sheath around ovarian cancer cells could promote the motility of metastatic CD44 expressing OVCAR-5 and

SKOV-3 cells, but not by low-metastatic OVCAR-3 cells which lack CD44. The motility of OVCAR-5 and SKOV-3 cells was significantly increased in scratch wound and chemotaxis assays following treatment with recombinant versican. We demonstrated that small HA oligosaccharides (6-10) were able to significantly block formation of pericellular sheath, motility, and invasion of OVCAR-5 cells following treatment with versican. Treatment with exogenous HA increased ovarian cancer cell adhesion to peritoneal cells, and this increase was successfully blocked by the addition of HA oligosaccharides or treatment of the LP-9 monolayer with hyaluronidase. These novel findings indicate that the acquisition of a HA-versican pericellular sheath by ovarian cancer cells may aid their peritoneal dissemination and metastasis. Our results suggest that HA oligosaccharides may be effective at inhibiting the invasion of CD44 positive ovarian cancers and warrants further study as a potential therapy.

Overall, the studies in this thesis indicate a very strong role for the tumour microenvironment, and in particular the proteolysis of proteins in the tumour microenvironment. Further investigation will increase our understanding of the mechanisms and pathways involved in the proteolytic cascade which is triggered during ovarian cancer metastasis.

DECLARATION

I certify that this thesis contains no material which has been accepted for the award of any other degree or diploma in any univeristy or other tertiary institution to Miranda Ween and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Ween MP, Hoffmann P, Rodgers RJ, Ricciardelli C, & Oehler MK. Transforming growth factor induced protein TGFBIp promotes ovarian cancer cell motility and adhesion to peritoneal cells. 5th International Conference on Tumour Microenvironment, Versailles, France, October 2009 (Selected for oral presentation).

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ABBREVIATIONS

ACN	–	Acetonitrile
ADAMTS	–	Adamalysin with Thrombospondin type 1 Motifs
AR	–	Androgen Receptor
BK	–	Bradykinin
CA125	–	Cancer Antigen 125
CD44s	–	Standard CD44
CD44v	–	CD44 variants
ChABC	–	Chondroitinase ABC
CK-1	–	Cytokeratin-1/Keratin Type II Cytoskeletal 1
CK-5	–	Cytokeratin-5/Keratin Type II Cytoskeletal 5
CK-6C	–	Cytokeratin-6C/Keratin Type II Cytoskeletal 6C
CK-9	–	Cytokeratin-9/Keratin Type I Cytoskeletal 9
CK-10	–	Cytokeratin-10/Keratin Type I Cytoskeletal 10
CK-14	–	Cytokeratin-14/Keratin Type I Cytoskeletal 14
CK-16	–	Cytokeratin-16/Keratin Type I Cytoskeletal 16
CM	–	Conditioned Medium
CS	–	Chondroitin Sulphate
C-Terminal Domain	–	Carboxy Terminus Domain
DB	–	Dilution Buffer
DCIS	–	Ductal carcinoma *in situ*
ε-ACA	–	6-Aminocaproic Acid
ECL	–	Enhanced Chemiluminesence
ECM	–	Extracellular Matrix
EGF	–	Epidermal Growth Factor
EHS	–	Engelbreth-Holm-Swarm
ELISA	–	Enzyme Linked Immunosorbent Assay
EMI	–	Emilin and Multimerin
EPHX	–	Epoxide Hydrolase
ER	–	Oestrogen Receptor
FAS	–	Fasciclin
FIGO	–	International Federation of Gynaecologists and Obstetricians
GM6001	–	Galardin
H & E	–	Haematoxylin and Eosin
HA	–	Hyaluronan
HAase	–	Hyaluronidase
HER	–	Human Epidermal Growth Factor Receptor
HK	–	High Molecular Weight Kininogen
HMEC	–	Human Mammary Epithelial Cell
IPG	–	Immobilized pH Gradient
KO	–	Knockout

LC-ESI	–	Liquid Chromatography-Electrospray Ionisation
LMP	–	Low Malignant Potential
MALDI-TOF/TOF	–	Matrix Assistant Laser Desorption/Ionisation Time of Flight/Time of Flight
MMP	–	Matrix Metalloproteinase
MPNST	–	Malignant Peripheral Nerve Sheath Tumour
MS	–	Mass Spectrometry
N-Terminal Domain	–	Amino Terminus Domain
Oligo	–	Oligosaccharide
OSE	–	Normal Ovarian Surface Epithelial Cells
PAGE	–	Polyacrylamide Gel Electrophoresis
PAI-1	–	Plasminogen Activator Inhibitor-1
PBCA	–	Poly Butyl Cyanoacrylate
PBS	–	Phosphate Buffered Saline
PBS-T	–	Phosphate Buffered Saline with 0.05% Tween-20
PDGF	–	Platelet Derived Growth Factor
PEI	–	Polyethylenimine
PI	–	Broad Spectrum Protease Inhibitor
PR	–	Progesterone Receptor
RAAS	–	Renin Angiotensin Aldosterone System
RAH	–	Royal Adelaide Hospital
RGD	–	Arg-Gly-Asp
RT	–	Room Temperature
rTGFBIp	–	Recombinant TGFBIp
rV1	–	Recombinant Versican Isoform V1
SDS	–	Sodium Dodecyl Sulphate
SMCs	–	Smooth Muscle Cells
SNP	–	Single Nucleotide Polymorphism
TGFβ	–	Transforming Growth Factor β
TGFBIp	–	Transforming Growth Factor Inducible Protein
TKT	–	Transketolase
tPA	–	Tissue Type Plasminogen Activator
uPA	–	Urokinase Type Plasminogen Activator
uPAR	–	Urokinase Plasminogen Activator Surface Receptor
VEGF	–	Vascular Endothelial Growth Factor