Assessment of plasminogen activator inhibitor type 2 (PAI-2) as an imaging and therapeutic agent of human cancer

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ASSESSMENT OF PLASMINOGEN ACTIVATOR INHIBITOR TYPE 2 (PAI-2) AS AN IMAGING AND THERAPEUTIC AGENT OF HUMAN CANCER

A thesis submitted in fulfilment of the requirements for the award of the degree

PhD

from

UNIVERSITY OF WOLLONGONG

by

MINH-THU NGUYEN HANG, B.Sc.(Hons)

DEPARTMENT OF BIOLOGICAL SCIENCES
2001
STATEMENT OF ORIGINALITY

I, Minh-Thu N. Hang, declare that this thesis contains no material which has been accepted for the award of any degree or diploma in any University, and to the best of my knowledge contains no material which has been previously published or written by another person except where due reference is made in the text of this thesis.

Minh-Thu N. Hang
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ABSTRACT

The plasminogen activation cascade is an important proteolytic pathway involved in the growth and spread of cancer. Potentially, an inhibitor of plasminogen activation could make an excellent cancer imaging agent or cancer treatment. The aim of this thesis was to assess whether plasminogen activator inhibitor 2 (PAI-2) can image or treat colorectal cancer. The first part of this thesis examined the ability of PAI-2 to bind specifically to the human colorectal cancer cell line HCT116. These experiments involved confirmation of u-PA expression by HCT116 cells and cell binding studies with $^{125}$I-PAI-2. The second part was examining the biodistribution and kinetics of $^{125}$I-PAI-2 in nude mice bearing tumour xenografts derived from HCT116 cells. The final part involved examining the effect PAI-2 treatment had on mice bearing HCT116 tumour xenografts.

PAI-2 was found to bind specifically to u-PA on HCT116 cells. There appeared to be a high turnover rate of bound PAI-2 because it was difficult to detect $^{125}$I-PAI-2/u-PA complexes by autoradiography. $^{125}$I-PAI-2 had a biphasic distribution in the bloodstream of control mice (distribution phase ($T_{1/2a}$) 12.5min, elimination phase ($T_{1/2b}$) 342min) and mice bearing tumour xenografts ($T_{1/2a}$ 1.4min, $T_{1/2b}$ 29min). Approximately 1% of $^{125}$I-PAI-2 localised to the tumour xenograft after a single intravenous injection. However, more $^{125}$I-PAI-2 could be localised to the tumour by multiple intravenous injections. From three separate therapy experiments with PAI-2, there did not appear to be any effect on relatively large tumours. However, in one
experiment PAI-2 injections did cause two 1mm tumours to disappear. In conclusion, PAI-2 does bind to u-PA on HCT116 cells *in vitro*. *In vivo*, injected PAI-2 appeared unsuitable for the imaging of tumours or metastasis. However preliminary data from this thesis suggest that PAI-2 may have therapeutic potential against smaller tumours.
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<th>Description</th>
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<tbody>
<tr>
<td>#387</td>
<td>t-PA monoclonal antibody</td>
</tr>
<tr>
<td>#394</td>
<td>u-PA monoclonal antibody</td>
</tr>
<tr>
<td>#3750</td>
<td>PAI-2 monoclonal antibody</td>
</tr>
<tr>
<td>#3936</td>
<td>u-PAR monoclonal antibody</td>
</tr>
<tr>
<td>API</td>
<td>Activator protein 1</td>
</tr>
<tr>
<td>ATTC</td>
<td>American Tissue Type Collection</td>
</tr>
<tr>
<td>B428</td>
<td>4-iodobenzo[b]thiopene-2-carboxamidine, a synthetic u-PA inhibitor</td>
</tr>
<tr>
<td>bFGF</td>
<td>Basic fibroblast growth factor</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>CEA</td>
<td>Carcinoembryonic antigen</td>
</tr>
<tr>
<td>DNP-9</td>
<td>IgG1 isotype control antibody</td>
</tr>
<tr>
<td>DTNB</td>
<td>5,5’-dithiobis (2-nitrobenzoic acid)</td>
</tr>
<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>EGF</td>
<td>Epidermal growth factor</td>
</tr>
<tr>
<td>EGR-CMK</td>
<td>Glu-Gly-Arg chloromethyl ketone</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme linked immunosorbent assay</td>
</tr>
<tr>
<td>ER</td>
<td>Estrogen receptor</td>
</tr>
<tr>
<td>FBS</td>
<td>Fetal bovine serum</td>
</tr>
<tr>
<td>FITC</td>
<td>Fluorescein isothiocyanate</td>
</tr>
<tr>
<td>G155-78</td>
<td>IgG2a isotype control antibody</td>
</tr>
<tr>
<td>GFD</td>
<td>Growth factor domain</td>
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<tr>
<td>GM-CSF</td>
<td>Granulocyte-macrophage colony stimulating factor</td>
</tr>
<tr>
<td>gp330</td>
<td>Glycoprotein 330</td>
</tr>
<tr>
<td>GPI</td>
<td>Glycosylphosphatidylinositol</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>Hemotoxylin &amp; Eosin</td>
</tr>
<tr>
<td>hPAI-2</td>
<td>Human plasminogen activator inhibitor type 2</td>
</tr>
<tr>
<td>HUVEC</td>
<td>Human umbilical vein endothelial cells</td>
</tr>
<tr>
<td>i.p.</td>
<td>Intraperitoneal</td>
</tr>
<tr>
<td>i.v.</td>
<td>Intravenous</td>
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<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>IL-1</td>
<td>Interleukin-1</td>
</tr>
<tr>
<td>IL-2</td>
<td>Interleukin-2</td>
</tr>
<tr>
<td>IUGR</td>
<td>Intra-uterine growth retardation</td>
</tr>
<tr>
<td>K_d</td>
<td>Dissociation constant</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>LRP</td>
<td>Low density lipoprotein receptor-related protein</td>
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<tr>
<td>M-CSF</td>
<td>Macrophage colony stimulating factor</td>
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<td>MMP-2</td>
<td>Matrix metalloproteinase 2</td>
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<tr>
<td>mPAI-2</td>
<td>Mouse plasminogen activator inhibitor type 2</td>
</tr>
<tr>
<td>Mr</td>
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OD  Optical density
OPD  o-Phenyldiamine
PA  Plasminogen activator
PAI  Plasminogen activator inhibitor
PAI-1  Plasminogen activator inhibitor type 1
PAI-2  Plasminogen activator inhibitor type 2
PAI-3  Plasminogen activator inhibitor type 3
PBS  Phosphate buffered saline
PEG  Polyethylene glycol
PGE2  Prostaglandin E2
PI  Propidium iodide
PKC  Protein kinase C
PLD  Phospholipase D
PMA  Phorbol myristate acetate
PMSF  Phenylmethylsulfoxide fluoride
PN-1  Protease nexin 1
$r^2$  Correlation coefficient
RAP  Receptor associated protein
RIA  Radioimmunoassay
RIGS  Radioimmunoguided surgery
RT  Room temperature
s.c.  Subcutaneous
sc-tPA  Single chain tissue type plasminogen activator
sc-uPA/pro-u-PA  Single chain urokinase type plasminogen activator
SCID  Severe combined immunodeficient
SD  Standard deviation
SDS-PAGE  Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SERPIN  Serine protease inhibitor
SLE  Systemic lupus erythematosus
TAG  Tumour associated glycoprotein
TBS  Tris buffered saline
tc-tPA  Twin chain tissue type plasminogen activator
tc-uPA  Twin chain urokinase type plasminogen activator
TCA  Trichloroacetic acid
TGF-α  Transforming growth factor α
TGF-β1  Transforming growth factor β1
TNF-α  Tumor necrosis factor alpha
TNP  Trinitrophenol
t-PA  Tissue type plasminogen activator
TSP-1  Thrombospondin 1
u-PA  Urokinase type plasminogen activator
u-PAR  Urokinase type plasminogen activator receptor
UV  ultra-violet
VEGF  Vascular endothelial growth factor
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>VLDL</td>
<td>Very low density lipoprotein receptor</td>
</tr>
<tr>
<td>ZLS</td>
<td>Z-lysine thibenzyl ester</td>
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