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

Minh-Thu Nguyen Hang
University of Wollongong

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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 ($T_{1/2\alpha}$) 12.5min, elimination phase ($T_{1/2\beta}$) 342min) and mice bearing tumour xenografts ($T_{1/2\alpha}$ 1.4min, $T_{1/2\beta}$ 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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ABBREVIATIONS

| | |
|----------------|--|
| #387 | t-PA monoclonal antibody |
| #394 | u-PA monoclonal antibody |
| #3750 | PAI-2 monoclonal antibody |
| #3936 | u-PAR monoclonal antibody |
| AP1 | Activator protein 1 |
| ATTC | American Tissue Type Collection |
| B428 | 4-iodobenzo[b]thiopene-2-carboxamidine, a synthetic u-PA inhibitor |
| bFGF | Basic fibroblast growth factor |
| BSA | Bovine serum albumin |
| CEA | Carcinoembryonic antigen |
| DNP-9 | IgG1 isotype control antibody |
| DTNB | 5,5'-dithiobis (2-nitrobenzoic acid) |
| ECM | Extracellular matrix |
| EDTA | Ethylenediaminetetraacetic acid |
| EGF | Epidermal growth factor |
| EGR-CMK | Glu-Gly-Arg chloromethyl ketone |
| ELISA | Enzyme linked immunosorbent assay |
| ER | Estrogen receptor |
| FBS | Fetal bovine serum |
| FITC | Fluorescein isothiocyanate |
| G155-78 | IgG2a isotype control antibody |
| GFD | Growth factor domain |
| GM-CSF | Granulocyte-macrophage colony stimulating factor |
| gp330 | Glycoprotein 330 |
| GPI | Glycosylphosphatidylinositol |
| H&E | Hemotoxylin & Eosin |
| hPAI-2 | Human plasminogen activator inhibitor type 2 |
| HUVEC | Human umbilical vein endothelial cells |
| i.p. | Intraperitoneal |
| i.v. | Intravenous |
| IgG | Immunoglobulin G |
| IL-1 | Interleukin-1 |
| IL-2 | Interleukin-2 |
| IUGR | Intra-uterine growth retardation |
| K _d | Dissociation constant |
| LPS | Lipopolysaccharide |
| LRP | Low density lipoprotein receptor-related protein |
| M-CSF | Macrophage colony stimulating factor |
| MMP-2 | Matrix metalloproteinase 2 |
| mPAI-2 | Mouse plasminogen activator inhibitor type 2 |
| M _r | Molecular weight |

| | |
|------------------|--|
| 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 |
| PGE ₂ | Prostaglandin E ₂ |
| PI | Propidium iodide |
| PKC | Protein kinase C |
| PLD | Phospholipase D |
| PMA | Phorbol myristate acetate |
| PMSF | Phenylmethanesulfonyl 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 |

VLDL
ZLS

Very low density lipoprotein receptor
Z-lysine thibenzyl ester

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