UNIVERSITI PUTRA MALAYSIA

ROLES OF TRANSFORMING GROWTH FACTOR-BETA, INSULIN-LIKE GROWTH FACTORS AND PROTEASES IN HUMAN BREAST CANCER

WONG SHEW FUNG

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By

WONG SHEW FUNG

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of the Requirements for the Degree of Master of Science

July 2002

Specially dedicated to,

My beloved parents, husband, brothers, supervisors and friends

For their invaluable support, love, patience and intellectual stimulation……

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

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WONG SHEW FUNG

July 2002

Chairman : Associate Professor Dr Seow Heng Fong

Faculty : Medicine and Health Sciences

Transforming growth factor-beta (TGFβ) is present in breast cysts, normal and cancerous breast tissue. It plays an important role in oestrogen metabolism. However, TGFβ is present predominantly in latent forms and the mechanisms by which latent TGFβ is activated physiologically remain largely an enigma.

The aims of this study were:-

1) to investigate the effects of $TGF\beta_1$, IGF-I and IGF-II on cell growth, E_1STS and E_2DH activities in the hormone-dependent MCF-7 and hormone-independent MDA-MB-231 human breast cancer cell lines.
2) to investigate the effect of Cathepsin D and PSA on $TGF\beta_1$ and $TGF\beta_2$ mRNA expression, and their protein levels in both of the cell lines.
3) to study the effect of Cathepsin D and PSA on Type 1 and Type 2 E_2DH mRNA expression, E_1 and E_2 levels in both cell lines.

$TGF\beta_1$, IGF-I and IGF-II either alone or in combination inhibited cell growth of both cell lines but no additive or synergistic effects were observed. These treatments significantly stimulated E_1STS activity in the MCF-7 cell line except for $TGF\beta_1$ alone and $TGF\beta_1$ and IGF-I in combination. Only $TGF\beta_1$ and IGF-II acted synergistically to stimulate E_1STS activity in the MCF-7 cells. There was no significant effect on E_1STS activity in the MDA-MB-231 cells with any of the treatments. In the MCF-7 cell line, $TGF\beta_1$ and IGF-I, IGF-I and IGF-II, and $TGF\beta_1$, IGF-I and IGF-II acted synergistically to stimulate reductive E_2DH activity while only $TGF\beta_1$, IGF-I and IGF-II synergistically stimulated oxidative E_2DH activity. There were no synergistic effects on both oxidative and reductive E_2DH activities in the MDA-MB-231 cell line.

Cathepsin D (200 ng/ml) induced $TGF\beta_1$ mRNA at 72 hours whilst Cathepsin D concentrations of 50, 100 and 200 ng/ml reduced $TGF\beta_2$ mRNA at 8 hours in the MCF-7 cell line. PSA (50 ng/ml) downregulated $TGF\beta_1$ and $TGF\beta_2$ mRNA at 72 and 8 hours, respectively. Cathepsin D (100 ng/ml) upregulated $TGF\beta_2$ mRNA at 24 hours in the MDA-MB-231 cell line. PSA (50, 200 and 400 ng/ml) induced $TGF\beta_1$ mRNA at 24 hours in the MDA-MB-231 cells. Both Cathepsin D and PSA were unable to activate latent $TGF\beta_1$ and $TGF\beta_2$ in both cell lines while a significant increase of active $TGF\beta_2$ throughout the experiment was observed. Cathepsin D and PSA had no effect on reductive and oxidative E_2DH mRNA in the MCF-7 cell line. Cathepsin D (50, 100 and 200 ng/ml) at 24 hours and 50 ng/ml at 4 hours upregulated reductive and oxidative E_2DH mRNA respectively in the MDA-MB-231 cell line. PSA (50 ng/ml) induced while higher concentrations reduced reductive

E_2DH mRNA at 24 hours. PSA (200 ng/ml and 400 ng/ml) upregulated oxidative E_2DH mRNA. Both Cathepsin D and PSA had no effect on E_1 level in both cell lines. Cathepsin D (50 ng/ml) increased E_2 levels at 4 and 72 hours whilst reducing E_2 levels at 24 hours compared with 4 hours in the MCF-7 cell line. PSA (200 ng/ml and 400 ng/ml) induced E_2 levels at 4 and 24 hours respectively. Cathepsin D (200 ng/ml) significantly increased E_2 levels at 24 and 72 hours compared with 8 hours in the MDA-MB-231 cell line. Cathepsin D (100 ng/ml) significantly increased E_2 levels at 72 hours versus 4 hours. PSA (200 ng/ml at 8 hours, 200 ng/ml and 400 ng/ml at 72 hours) significantly increased E_2 levels compared with 4 hours. PSA (200 ng/ml and 400 ng/ml) reduced E_2 levels at 4 hours compared with 4 hours.

In conclusion, $TGF\beta_1$, IGF-I and IGF-II may exert synergistic effects on oestrogen metabolism especially in the MCF-7 cell line where they synergistically stimulated the conversion of E_1S to E_1 and E_1 to E_2. Cathepsin D and PSA were unable to activate latent $TGF\beta_1$ and $TGF\beta_2$. Both proteases may regulate the expression of $TGF\beta_1$ mRNA, $TGF\beta_2$ mRNA and E_2 protein in the MCF-7 cell line. They may regulate the expression of $TGF\beta_1$ mRNA, $TGF\beta_2$ mRNA, E_2 protein, reductive and oxidative E_2DH mRNA in the MDA-MB-231 cell line.

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PERANAN FAKTOR TRNAFORMASI PERTUMBUHAN-BETA, FAKTOR INSULIN-LIKE PERTUMBUHAN DAN PROTEASE DI KANSER PAYUDARA MANUSIA

Oleh

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Fakulti : Perubatan dan Sains Kesihatan

Faktor transformasi pertumbuhan-beta (TGFβ) terdapat di sis payudara, tisu payudara yang normal and kanser. Ia memainkan peranan yang penting dalam metabolisme estrogen. Namun demikian, kebanyakan TGFβ terdapat dalam bentuk laten dan mekanisme di mana laten TGFβ diaktifkan secara fisiologi masih belum diketahui.

Objektif penyelidikan ini ialah :-

1) untuk mengkaji kesan $TGF\beta_1$, IGF-I dan IGF-II terhadap pertumbuhan sel, E_1STS dan E_2DH aktiviti di jujukan sel kanser payudara manusia yang bergantung kepada hormon MCF-7 dan tidak bergantung kepada hormon MDA-MB-231.

2) untuk mengkaji kesan Cathepsin D dan PSA terhadap $TGF\beta_1$ dan $TGF\beta_2$ mRNA ekspresi, dan paras protein masing-masing di kedua-dua jujukan sel tersebut.

3) untuk mengkaji kesan Cathepsin D dan PSA terhadap Jenis 1 dan Jenis 2 E_2DH mRNA ekspresi, paras E_1 dan E_2 di kedua-dua jujukan sel tersebut.

TGFβ_1, IGF-I dan IGF-II secara persendirian atau dalam kombinasi merencatkan pertumbuhan sel bagi kedua-dua jujukan sel tetapi tiada kesan tambahan atau sinergistik diperhatikan. Rawatan ini merangsang E_1STS aktiviti secara signifikan di jujukan sel MCF-7 kecuali bagi TGFβ_1 sahaja dan TGFβ_1 dan IGF-I secara kombinasi. Hanya TGFβ_1 dan IGF-II merangsang E_1STS aktiviti secara sinergistik di sel MCF-7. Kesemua rawatan ini tidak mempunyai kesan terhadap E_1STS aktiviti di sel MDA-MB-231. Di jujukan sel MCF-7, TGFβ_1 dan IGF-I, IGF-I dan IGF-II, dan TGFβ_1, IGF-I dan IGF-II mempercepatkan reduktif E_2DH secara sinergistik meskipun hanya TGFβ_1, IGF-I dan IGF-II sahaja dapat mempercepatkan oksidatif E_2DH aktiviti secara sinergistik. Tiada kesan sinergistik diperhatikan pada oksidatif and reduktif E_2DH aktiviti di jujukan sel MDA-MB-231.

Cathepsin D (200 ng/ml) meningkatkan TGFβ_1 mRNA pada 72 jam meskipun mengurangkan TGFβ_2 mRNA pada 8 jam bagi 50, 100 dan 200 ng/ml di jujukan sel MCF-7. PSA (50 ng/ml) mengurangkan TGFβ_1 dan TGFβ_2 mRNA pada 72 jam dan 8 jam masing-masing. Cathepsin D (100 ng/ml) meningkatkan TGFβ_2 mRNA pada 24 jam di jujukan sel MDA-MB-231. PSA (50, 200 dan 400 ng/ml) meningkatkan TGFβ_1 mRNA pada 24 jam di sel MDA-MB-231. Kedua-dua Cathepsin D dan PSA tidak dapat mengaktifkan laten TGFβ_1 dan TGFβ_2 di kedua-dua jujukan sel tersebut meskipun terdapat peningkatan TGFβ_2 yang aktif secara signifikan sepanjang tempoh eksperimen. Cathepsin D dan PSA tidak mempunyai kesan ke atas reduktif dan oksidatif E_2DH mRNA di jujukan sel MCF-7. Cathepsin D (50, 100 dan 200

ng/ml) pada 24 jam dan 50 ng/ml pada 4 jam meningkatkan reduktif dan oksidatif E_2DH mRNA masing-masing di jujukan sel MDA-MB-231. PSA (50 ng/ml) meningkatkan meskipun kepekatan tinggi mengurangkan reduktif E_2DH mRNA pada 24 jam. PSA (200 dan 400 ng/ml) meningkatkan oksidatif E_2DH mRNA. Kedua-dua Cathepsin D dan PSA tidak mempunyai kesan terhadap paras E_1 di kedua-dua jujukan sel tersebut. Cathepsin D (50 ng/ml) meninggikan paras E_2 pada 4 dan 72 jam meskipun mengurangkan paras E_2 pada 24 jam berbanding dengan 4 jam di jujukan sel MCF-7. PSA (200 dan 400 ng/ml) meningkatkan paras E_2 pada 4 dan 24 jam masing-masing. Cathepsin D (200 ng/ml) meninggikan paras E_2 secaa signifikan pada 24 dan 72 jam berbanding dengan 8 jam di jujukan sel MDA-MB-231. Cathepsin D (100 ng/ml) meninggikan paras E_2 secara signifikan pada 72 jam berbanding dengan 4 jam. PSA (200 ng/ml pada 8 jam, 200 dan 400 ng/ml pada 72 jam) meningkatkan paras E_2 secara signifikan berbanding dengan 4 jam. PSA (200 dan 400 ng/ml) mengurangkan paras E_2 pada 4 jam berbanding dengan 4 jam.

Kesimpulannya, $TGF\beta_1$, IGF-I dan IGF-II boleh memberi kesan sinergistik ke atas estrogen metabolisme terutamanya di jujukan sel MCF-7 di mana mereka merangsang pertukaran E_1S kepada E_1 dan E_1 kepada E_2. Cathepsin D dan PSA tidak dapat mengaktifkan laten $TGF\beta_1$ dan $TGF\beta_2$. Kedua-dua protease mungkin mempengaruhi ekspersi $TGF\beta_1$ mRNA, $TGF\beta_2$ mRNA dan protein E_2 di jujukan sel MCF-7. Mereka juga mempengaruhi ekspresi $TGF\beta_1$ mRNA, $TGF\beta_2$ mRNA, protein E_2, reduktif dan oksidatif E_2DH mRNA di jujukan sel MDA-MB-231.

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirements for the Degree of Master of Science. The members of the Supervisory Committee are as follows:

Dr Seow Heng Fong, Ph.D.
Associate Professor,
Faculty of Medicine and Health Sciences,
Universti Putra Malaysia.
(Chairperson)

Dr Leslie Charles Lai Chin Loy, MBBS, MSc, MD, FRCP, FRCPath, FAMM.
Professor,
Postgraduate Studies,
International Medical University.
(Member)

Dr Rozita Rosli, Ph.D.
Faculty of Medicine and Health Sciences,
Universiti Putra Malaysia.
(Member)

AINI IDERIS, Ph.D.
Professor / Dean
School of Graduate studies,
Universiti Putra Malaysia

Date:

DECLARATION FORM

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

WONG SHEW FUNG

Date:

TABLE OF CONTENTS

		Page
DEDICATION		**ii**
ABSTRACT		**iii**
ABSTRAK		**iv**
ACKNOWLEDGEMENTS		**v**
APPROVAL SHEETS		**vi**
DECLARATION FORM		**vii**
LIST OF TABLES		**xv**
LIST OF FIGURES		**xvii**
LIST OF ABBREVIATIONS		**xxi**
CHAPTER		
I	**INTRODUCTION**	**1**
	1.1 General Overview	1
	1.2 Hypotheses and Objectives	3
II	**LITERATURE REVIEW**	**5**
	2.1 Breast Cancer in the World	5
	2.2 Breast Cancer in Malaysia	6
	2.3 Transforming Growth Factor-Beta (TGFβ)	8
	2.3.1 Molecular Structure	8
	2.3.2 Mechanism of Activation	11
	2.3.3 TGFβ Signalling Pathway	12
	2.3.4 Biological Effects of TGFβ	13
	2.3.5 TGFβ and Breast Cancer	17
	2.4 Insulin-like Growth Factor (IGF)	19
	2.4.1 Molecular Structure	19
	2.4.2 IGF Signalling Pathway	21
	2.4.3 Biological Functions of IGF	22
	2.4.4 IGF and Breast Cancer	22
	2.5 Proteases	25
	2.5.1 Cathepsin D	25
	2.5.2 PSA	31
	2.6 Oestrogen and Breast Cancer	35
	2.6.1 Detection and Measurement of Oestrogen and Metabolites in Breast Cancer	35
	2.6.2 Effects of TGFβ on Oestrogen Metabolism in Breast Cancer	40
	2.6.3 Effects of IGF on Oestrogen Metabolism in Breast Cancer	40
	2.6.4 Effects of Cathepsin D and PSA on Oestrogen Metabolism	41
III	**MATERIALS AND METHODS**	**42**
	3.1Materials	42
	3.1.1 Chemicals	42
	3.1.2 Cell Culture	42
	3.1.3 Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)	43
	3.1.4 ELISA	43
	3.1.5 Reagents	44

3.2 Methods 44
3.2.1 Stripping of FBS 44
3.2.2 Freezing and Thawing of Cells 45
3.2.3 Cell Culture 45
3.2.4 Experimental Procedure to Investigate the Effect of TGFβ, IGF-I and IGF-II on Growth, E_1STS and E_2DH Activity 46
3.2.5 Oestrone Sulphatase (E_1STS) Assay 48
3.2.6 Oestradiol-17β Hydroxysteroid Dehydrogenase (E_2DH) Assay 50
3.2.7 Determination of Cell Numbers 52
3.2.8 RT-PCR 52
3.2.9 ELISA 56
3.2.10 Statistical Analysis 58

IV RESULTS 59
4.1 Effects of TGFβ and IGF on Cell Growth 59
4.2 Effects of TGFβ and IGF on E_1STS Assay 61
4.3 Effects of TGFβ and IGF on E_2DH activity 63
4.4 *In Vitro* activation of Latent TGFβ 67
4.4.1 Effect of Cathepsin D and PSA on TGFβ_1 mRNA Expression 67
4.4.2 Effect of Cathepsin D and PSA on TGFβ_2 mRNA Expression 75
4.4.3 Effect of Cathepsin D and PSA on GAPDH mRNA Expression 83
4.4.4 Effect of Cathepsin D and PSA on TGFβ_1 level 88
4.4.5 Effect of Cathepsin D and PSA on TGFβ_2 level 89
4.5 Effects of Cathepsin D and PSA on E_2DH activity 91
4.5.1 Effect of Cathepsin D and PSA on reductive E_2DH mRNA Expression 91
4.5.2 Effect of Cathepsin D and PSA on Oxidative E_2DH mRNA Expression 99
4.5.3 Effect of Cathepsin D and PSA on E_1 Level 101
4.5.4 Effect of Cathepsin D and PSA on E_2 Level 102

V DISCUSSION 107
VI CONCLUSION AND RECOMMENDATIONS 125

REFERENCES 128
APPENDICES 151
BIODATA OF THE AUTHOR 161
PUBLICATIONS 162

LIST OF TABLES

Table		Page
1	Estimated breast cancer cases and deaths world wide in 2000.	6
2	Cases of Benign and Malignant Breast Diseases seen in Breast Clinics, 1995.	7
3	Biological effects of TGFβ (Flanders and Roberts, 2001).	16
4	Quantities of DNA fragments in 0.3333 μg GeneRuler 100 bp DNA Ladder.	56
5	Effect of pH on activation of latent TGFβ in chicken embryo fibroblast conditioned media (Adapted from Lawrence *et al.*, 1985).	118
6	The effects of $TGF\beta_1$, IGF-I and IGF-II on cell growth, E_1STS, reductive and oxidative E_2DH activities in the MCF-7 breast cancer cell line.	155
7	The effects of $TGF\beta_1$, IGF-I and IGF-II on cell growth, E_1STS, reductive and oxidative E_2DH activities in the MDA-MB-231 breast cancer cell line.	156
8	The effects of Cathepsin D on $TGF\beta_1$, $TGF\beta_2$, GAPDH, reductive and oxidative E_2DH mRNA expression in the MCF-7 breast cancer cell line.	157
9	The effects of Cathepsin D on $TGF\beta_1$, $TGF\beta_2$, E_1 and E_2 levels in the MCF-7 breast cancer cell line.	157
10	The effects of PSA on $TGF\beta_1$, $TGF\beta_2$, GAPDH, reductive and oxidative E_2DH mRNA expression in the MCF-7 breast cancer cell line.	158
11	The effects of PSA on $TGF\beta_1$, $TGF\beta_2$, E_1 and E_2 levels in the MCF-7 breast cancer cell line.	158
12	The effects of Cathepsin D on $TGF\beta_1$, $TGF\beta_2$, GAPDH, reductive and oxidative E_2DH mRNA expression in the MDA-MB-231 breast cancer cell line.	159
13	The effects of Cathepsin D on $TGF\beta_1$, $TGF\beta_2$, E_1 and E_2 levels in the MDA-MB-231 breast cancer cell line.	159

14	The effects of PSA on $TGF\beta_1$, $TGF\beta_2$, GAPDH, reductive and oxidative E_2DH mRNA expression in the MDA-MB-231 breast cancer cell line.	160
15	The effects of PSA on $TGF\beta_1$, $TGF\beta_2$, E_1 and E_2 levels in the MDA-MB-231 breast cancer cell line.	160

LIST OF FIGURES

Figure		Page
1	Synthesis and secretion of TGFβ (Modified from Clark and Coker, 1998).	10
2	The TGFβ signalling pathway.	12
3	Cell adhesion molecules whose expression is regulated by TGFβ (Adapted from Massague J, 1990).	15
4	IGF signalling pathway (Modified from Jones and Clemmons, 1995).	21
5	Oestrogen metabolism pathway.	37
6	Effect of TGFβ, IGF-I and IGF-II on Growth, E_1STS and E_2DH Activity.	47
7	Flow chart of the E_1STS assay.	49
8	Flow chart of E_2DH assay.	51
9	Effects of $TGF\beta_1$ (4 ng/ml), IGF-I (200 ng/ml) and IGF-II (200 ng/ml) on cell growth of (a) MCF-7 and (b) MDA-MB-231 breast cancer cells. Cell counts are expressed as means of triplicate determinations plus 1 SD. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ vs. control.	60
10	Effects of $TGF\beta_1$ (4 ng/ml), IGF-I (200 ng/ml) and IGF-II (200 ng/ml) on E_1STS activity in intact (a) MCF-7 (b) MDA-MB-231 breast cancer cell lines. Values shown are means of triplicate determinations plus 1 SD. $^{*}P < 0.05$; $^{***}P < 0.001$ vs. control.	62
11	Effects of $TGF\beta_1$ (4 ng/ml), IGF-I (200 ng/ml) and IGF-II (200 ng/ml) on reductive E_2DH activity ($E_1 \rightarrow E_2$) in the (a) MCF-7 (b) MDA-MB-231 breast cancer cell lines. Values shown are means of triplicate determinations plus 1 SD. $^{*}P < 0.05$; $^{**}P < 0.01$ vs. control.	65
12	Effects of $TGF\beta_1$ (4 ng/ml), IGF-I (200 ng/ml) and IGF-II (200 ng/ml) on oxidative E_2DH activity ($E_2 \rightarrow E_1$) in the (a) MCF-7 (b) MDA-MB-231 breast cancer cell lines. Values are expressed as means of triplicate determinations plus 1 SD. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ vs. control.	66

13	Representative RT-PCR for $TGF\beta_1$ mRNA expression after (a) 4 hours, (b) 8 hours, (c) 24 hours and (d) 72 hours post Cathepsin D (Lane 2-6) and PSA (Lane 7-11) in the MCF-7 breast cancer cell line.	68
14	SynGene Gene Tool analysis of $TGF\beta_1$ mRNA expression profile post (a) Cathepsin D and (b) PSA treatment in the MCF-7 breast cancer cell line.	69
15	Expression of $TGF\beta_1$ mRNA relative to GAPDH mRNA in the MCF-7 breast cancer cell line after (a) Cathepsin D and (b) PSA treatment.	70
16	Representative RT-PCR for $TGF\beta_1$ mRNA expression after (a) 4 hours, (b) 8 hours, (c) 24 hours and (d) 72 hours post Cathepsin D (Lane 2-6) and PSA (Lane 7-11) in the MDA-MB-231 breast cancer cell line.	72
17	SynGene Gene Tool analysis of $TGF\beta_1$ mRNA expression profile post (a) Cathepsin D and (b) PSA treatment in the MDA-MB-231 breast cancer cell line.	73
18	Expression of $TGF\beta_1$ mRNA relative to GAPDH mRNA in the MDA-MB-231 breast cancer cell line after (a) Cathepsin D and (b) PSA treatment.	74
19	Representative RT-PCR for $TGF\beta_2$ mRNA expression after (a) 4 hours, (b) 8 hours, (c) 24 hours and (d) 72 hours post Cathepsin D (Lane 2-6) and PSA (Lane 7-11) in the MCF-7 breast cancer cell line.	76
20	SynGene Gene Tool analysis of $TGF\beta_2$ mRNA expression profile post (a) Cathepsin D and (b) PSA treatment in the MCF-7 breast cancer cell line.	77
21	Expression of $TGF\beta_2$ mRNA relative to GAPDH mRNA in the MCF-7 breast cancer cell line after (a) Cathepsin D and (b) PSA treatment.	78
22	Representative RT-PCR for $TGF\beta_2$ mRNA expression after (a) 4 hours, (b) 8 hours, (c) 24 hours and (d) 72 hours post Cathepsin D (Lane 2-6) and PSA (Lane 7-11) in the MDA-MB-231 breast cancer cell line.	80
23	SynGene Gene Tool analysis of $TGF\beta_2$ mRNA expression profile post (a) Cathepsin D and (b) PSA treatment in the MDA-MB-231 breast cancer cell line.	81

24	Expression of $TGF\beta_2$ mRNA relative to GAPDH mRNA in the MDA-MB-231 breast cancer cell line after (a) Cathepsin D and (b) PSA treatment.	82
25	Representative RT-PCR for GAPDH mRNA expression after (a) 4 hours, (b) 8 hours, (c) 24 hours and (d) 72 hours post Cathepsin D (Lane 2-6) and PSA (Lane 7-11) in the MCF-7 breast cancer cell line.	84
26	SynGene Gene Tool analysis of GAPDH mRNA expression profile post (a) Cathepsin D and (b) PSA treatment in the MCF-7 breast cancer cell line.	85
27	Representative RT-PCR for GAPDH mRNA expression after (a) 4 hours, (b) 8 hours, (c) 24 hours and (d) 72 hours post Cathepsin D (Lane 2-6) and PSA (Lane 7-11) in the MDA-MB-231 breast cancer cell line.	86
28	SynGene Gene Tool analysis of GAPDH mRNA expression profile post (a) Cathepsin D and (b) PSA treatment in the MDA-MB-231 breast cancer cell line.	87
29	A typical standard curve for $TGF\beta_1$ immunoassay.	88
30	A typical standard curve for $TGF\beta_2$ immunoassay.	89
31	Effect of (a) Cathepsin D and (b) PSA on $TGF\beta_2$ level in the MDA-MB-231 breast cancer cell line. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ vs. 4 hours; *** $P < 0.001$, **$P < 0.01$, *$P < 0.05$ vs 8 hours; ***$P < 0.001$, **$P < 0.01$ vs 24 hours.	90
32	Representative RT-PCR for reductive E_2DH mRNA expression after (a) 4 hours, (b) 8 hours, (c) 24 hours and (d) 72 hours post Cathepsin D (Lane 2-6) and PSA (Lane 7-11) in the MCF-7 breast cancer cell line.	92
33	SynGene Gene Tool analysis of reductive E_2DH mRNA expression profile post (a) Cathepsin D and (b) PSA treatment in the MCF-7 breast cancer cell line.	93
34	Expression of Reductive E_2DH mRNA relative to GAPDH mRNA in the MCF-7 breast cancer cell line after (a) Cathepsin D and (b) PSA treatment.	94
35	Representative RT-PCR for reductive E_2DH mRNA expression after (a) 4 hours, (b) 8 hours, (c) 24 hours and (d) 72 hours post Cathepsin D (Lane 2-6) and PSA (Lane 7-11) in the MDA-MB-231 breast cancer cell line.	96

36	SynGene Gene Tool analysis of reductive E_2DH mRNA expression profile post (a) Cathepsin D and (b) PSA treatment in the MDA-MB-231 breast cancer cell line.	97
37	Expression of Reductive E_2DH mRNA relative to GAPDH mRNA in the MDA-MB-231 breast cancer cell line after (a) Cathepsin D and (b) PSA treatment.	98
38	Representative RT-PCR for oxidative E_2DH mRNA expression after (a) 4 hours, (b) 8 hours, (c) 24 hours and (d) 72 hours post Cathepsin D (Lane 2-6) and PSA (Lane 7-11) in the MCF-7 breast cancer cell line.	99
39	Representative RT-PCR for oxidative E_2DH mRNA expression after (a) 4 hours, (b) 8 hours, (c) 24 hours and (d) 72 hours post Cathepsin D (Lane 2-6) and PSA (Lane 7-11) in the MDA-MB-231 breast cancer cell line.	100
40	A typical calibration curve for E_1 ELISA.	101
41	A typical standard curve for E_2 ELISA.	102
42	Effect of (a) Cathepsin D and (b) PSA on E_2 level in the MCF-7 breast cancer cell line. *$P < 0.05$ vs. 4 hours; *$P < 0.05$ vs control at 4 hours; *$P < 0.05$ vs control at 72 hours; ***$P < 0.001$ vs control at 24 hours.	104
43	Effect of (a) Cathepsin D and (b) PSA on E_2 level in the MDA-MB-231 breast cancer cell line. *$P < 0.05$; **$P < 0.01$ vs. 4 hours; *$P < 0.05$, **$P < 0.01$ vs control at 4 hours; *$P < 0.05$, **$P < 0.01$ vs 8 hours.	106
44	BLAST analysis of the sequence of the (a) forward and (b) reverse primers of the Type 1 or Reductive E_2DH compared with the published sequence.	154

LIST OF ABBREVIATIONS

μg/L	microgram per litre
μl	microlitre
^{14}C	carbon-14
^{35}S	sulphur-35
^{3}H	tritium
ACTH	adrenocorticotropic hormone
ATCC	American Type Tissue Culture
bFGF	basic fibroblast growth factor
BLAST	basic local alignment search tool
bp	base pair
cAMP	cyclic adenosine monophosphate
CDK	cyclin dependent kinase
cDNA	complementary deoxyribose nucleic acid
CO_2	carbon dioxide
DEPC	diethylpyrocarbonate
DHEA	dehydroepiandrosterone
DMEM	Dulbecco's Modified Eagle Medium
DMSO	dimethyl sulphoxide
DNA	deoxyribose nucleic acid
dNTP	PCR nucleotide mix
dpm	disintegrations per minute
E_1	oestrone
E_1S	oestrone sulphate
E_1STS	oestrone sulphatase

E_2	oestradiol
E_2DH	oestradiol-17β hydroxysteroid dehydrogenase
E_3	oestriol
EDTA	ethylenediamine tetracetic acid
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
EIA	enzyme immunoassay
ELISA	enzyme-linked immunoabsorbent assay
Endo F	endoglycosidase F
ER	oestrogen receptor
FBS	foetal bovine serum
FGF	fibroblast growth factor
FSH	follicle stimulating hormone
g	gram
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GC	Guanine - Cytosine
GS	Glycine - Serine
h	hours
HEPES	N-2-hydroxylpiperazine-N'-2-ethanesulphonic acid
His	histidine
HSP	heat shock protein
i.e.	that is
IFNγ	interferon gamma
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor binding protein

IGF-II/M6PR	insulin-like growth factor-II or mannose-6-phosphate receptor
IGF-IIR	insulin-like growth factor-II receptor
IGF-IR	insulin-like growth factor-I receptor
IL	interleukin
IRMA	immunoradiometric assay
K	potassium
kb	kilobase
kDa	kilo Dalton
M	molar
MEM	Minimal Essential Medium
mg/ml	milligram per millilitre
$MgCl_2$	magnesium chloride
ml	millilitre
M-MLV	Moloney Murine Leukemia Virus
mRNA	messenger ribonucleic acid
MW	molecular weight
N	normality
Na	sodium
NAD	nicotinamide adenine dinucleotide
NADPH	reduced nicotinamide adenine dinucleotide phosphate
ng/ml	nanogram per millilitre
nm	nanometre
NRK	normal rat kidney
p15	p15 / INK 4B
p21	p21 / WAF 1 / CIP 1

p27	p27 / kip 1
PAI	plasminogen activator inhibitor
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PDGF	platelet-derived growth factor
pg/ml	picogram per millilitre
pM	picomole
pRB	retinoblastoma gene product
PSA	prostate specific antigen
RGD	Arginine - Glycine - Aspartate
rpm	revolutions per minute
RT	reverse transcriptase
RT-PCR	reverse transcriptase polymerase chain reaction
SDS	sodium dodecyl sulphate
sec.	seconds
Ser	serine
SGF	sacroma growth factor
Smad	Sma or Mad related protein
TβR-I	transforming growth factor-beta type I receptor
TβR-II	transforming growth factor-beta type II receptor
TAE	Tris-acetate-EDTA
TGFα	transforming growth factor-alpha
TGFβ	transforming growth factor-beta
TIMP	tissue inhibitor of metalloproteinase
TLC	thin layer chromatography