Characterisation of Mammalian Cell Lines for use in Proliferative and Estrogenic Response Assays

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A thesis submitted in fulfilment of the requirements for the

Degree of Doctor of Philosophy at Flinders University

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2011

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Summary

Characterisation of Mammalian Cell Lines for use in Proliferative and Estrogenic Response Assays

A significant number of endocrine disrupting chemicals (EDCs), which have the ability to act as estrogens or disrupt the estrogen signalling pathway in humans and other species, have been reported in environmental waters,. Bioassays using human cell lines, which contain endogenous or inserted estrogen receptors, are useful to rapidly assess this (xeno)estrogen content of water.

This thesis presents a comparative assessment of the suitability of MCF7, ZR-75-1, H23, RL95-2, Ovcar and T-47D cell lines for (xeno)estrogen testing in a 96 well plate format. Cell lines were assessed for attachment time, proliferation rate, optimal time for producing a standard curve relating absorbance to cell number and the expression of ERα and ERβ mRNA and protein. Six hours was the optimum time for attachment and gave the smallest 95% confidence interval (CI) for a standard curve for MCF7, Ovcar, RL95-2 and T-47D cells. ZR-75-1 and H23 cells were best after 4 hours of attachment. Incubation for 24 hours gave the highest 95% CI and produced the least accurate standard curve. The reporting of a single doubling time for each cell line may not be accurate as cell lines had a doubling time that was density related. ERα expression was greatest in MCF7, followed by T-47D and ZR-75-1. ERβ was not detected in any cell line.

MCF7 cells are characterised as proliferating in response to (xeno)estrogens primarily via the ERα. The E-screen was originally developed using MCF7 cells, however, many modifications have lead to only semi-standardisation of the methodology. T-47D and ZR-75-1 cells have also been shown to proliferate in response to estrogen and were compared to MCF7 cells in an E-screen assay. The responses of cells using a 4hr or 6hr or an overnight attachment period in estrogen-free pre-treatment before exposure to estrogen for 3, 5 or 7 days were compared. The pre-treatment/attachment period did not affect EC_{50} values, however the proliferation of MCF7 cells almost doubled and the 95% CI was greatly reduced by reducing the attachment/ pre-treatment time to 6 hours. MCF7 cells are used in the E-screen

within a given passage range. The changes in sensitivity and response to estrogen of the three cell lines after extended time in culture (>18 passages) were also assessed. Although each cell line maintained similar EC_{50} values, the proliferative response decreased and variability increased.

The regulation of individual genes by estrogen has been widely studied. VEGF, IGFBP-4, pS2, MaoA and EGR1 were selected from microarray and *in vivo* data based on the magnitude and speed of response to estrogen exposure. The promoter regions, inclusive of their estrogen responsive elements, were identified. pS2 and MaoA promoter regions were successfully amplified and inserted into the pGL4.21(luc2P/Puro) reporter vector. Stable integration into T-47D cells resulted in non-estrogen inducible expression of the luciferase gene. Transient transfection of the vector into MCF7, ZR-75-1 and T-47D cells also resulted in expression of luciferase that could not be induced by estrogen.

Declaration

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in and university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Signed

Daniel Inglis

Acknowledgements

I sincerely thank Drs. Fiona Young and Andrew Humpage for their guidance, support and enthusiasm towards this project. With their supervision and critique, I have been able to contribute my small piece towards the field of Environmental Toxicology.

I also thank Dr Suzanne Froscio for reviewing earlier manuscripts and providing additional guidance. Thank you also to Dr George Mayne for guiding me in the use and analysis of real-time PCR.

Thanks also to all those in the Department of Medical Biotechnology and at the Australian Water Quality Centre for their support and contribution during this project, with special mention to Dina Zebian, Vicki Edwards, Rhys Hamon, Roshini Somashekar and visiting students. Also many thanks to Professor Dayanthi Nugegoda for her entertaining and educational visiting fellowship.

I would like to acknowledge the Australian Research Council (ARC) and SA Water for their generous financial support towards this project and its completion.

Finally, I would like to express my deepest gratitude towards my family who have supported me throughout this project, with patience and understanding.

List of Abbreviations

Amp	Ampicillin
ANOVA	Analysis of variance
AR	Androgen receptor
ATCC	American type culture collection
AU	Absorbance unit
bp	Base pairs
BPA	Bisphenol A
CHX	Cycloheximide
CI	Confidence interval
CPS	Counts per second
Cq	Quantification cycle
DBD	Deoxyribonucleic acid binding domain
DC-FCS	Dextran-charcoal treated fetal calf serum
DMEM	Dulbecco's modified Earles' medium
DNA	Deoxyribonucleic acid
E_2	17β-estradiol
EC_{50}	Effective concentration for half-maximal effect
EDC	Endocrine disrupting chemical
eGFP	Enhanced green fluorescent protein
ER	Estrogen receptor
ERE	Estrogen response element
EtBr	Ethidium bromide
EtOH	Ethanol
FCS	Fetal calf serum
GCMS	Gas chromatography / mass spectrometry
GWRC	Global water research coalition
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethansulphonic acid
Hsp	Heat shock protein
kDa	kilodalton
LB	Luria broth
LBD	Ligand binding domain
LOD	Limit of detection

Luc	Luciferase
mRNA	Messenger ribonucleic acid
MTT	3-(4,5-dimehtylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
OD	Optical density
PCR	Polymerase chain reaction
PE	Proliferative effect
PR	Phenol red
RIA	Radioimmunoassay
RPE	Relative proliferative effect
RPP	Relative proliferative potency
RT	Reverse transcription
RT-PCR	Reverse transcription real-time polymerase chain reaction
SD	Standard deviation
SEM	Standard error of mean
SOC	Super optimal broth with catabolite repression
SPE	Solid phase extraction
SRB	Sulforhodamine B
STP	Sewage treatment plant
TSS	Transcription start site
UTR	Untranslated region
UV	Ultraviolet
YES	Yeast estrogen screen

Publications and Conference Proceedings

Publications

- Characterisation of Mammalian Cell Lines for use in Proliferative and Estrogenic Response assays. Daniel J Inglis, Andrew R Humpage, Suzanne M Froscio, George C Mayne, Fiona M Young. (*Under Review*)

Conference Proceedings

- Flinders Research Centre for Coastal and Catchment Environments and Flinders University Bioknowledge Research Group Postgraduate Research Conference.. 18th-19th July 2006. Adelaide, Australia. *Oral Presentation "Development of a bioassay for measuring xenoestrogens in recycled water"*

- Society For Reproductive Biology 38th Annual Conference. 2nd-5th September 2007. Christchurch, New Zealand. *Oral Presentation "Development of an improved E-screen"*

- The Endocrine Society of Australia 51st Annual Scientific Meeting.. 25th – 28th August 2008. Melbourne, Australia. *Poster Presentation " A comparative Assessment of three cell lines for use in the E-screen"*

- Society of Environmental Toxicology and Chemistry World Congress. 3rd-7th August 2008. Sydney, Australia. *Oral Presentation "Improving the E-Screen: Effect of FCS Concentration on ZR75-1 Cell Proliferation and Estrogen Receptor Expression"*

- 2nd Australian Symposium on Ecological Risk Assessment and Management of Endocrine Disrupting Chemicals (EDCs), Pharmaceuticals and Personal Care Products (PPCPs) in the Australasian Environment. November 21st-22nd 2007. Canberra, Australia. *Poster Presentation " Development of an Improved E-Screen"*

- 13th Australasian Society for Ecotoxicology Conference: Toxicants in a changing environment. 20-23 September 2009. Adelaide, Australia. *Oral Presentation "Passage effect on MCF-7 and T-47D cells for use in the E-screen"*