

Mathematical Models of Cell Cycle Progression: Applications to Breast Cancer Cell Lines

Kate T. Simms

*Thesis submitted for the degree of
Doctor of Philosophy
in
Applied Mathematics
at
The University of Adelaide*

Discipline of Applied Mathematics, School of Mathematical Sciences,
Faculty of Engineering, Computer and Mathematical Sciences



June 16, 2012

Contents

Abstract	13
1 Introduction	15
1.1 Thesis summary	16
2 Biological background	21
2.1 The cell structure	21
2.2 Cancer is the result of mutations in DNA	25
Causes of mutations	25
2.3 Cancers come in different stages	26
2.4 The typical hallmarks of cancer	29
Hallmark 1: Self-sufficiency of growth signals	29
Hallmark 2: Insensitivity to anti-growth signals	30
Hallmark 3: Evasion of cell death signals	30
Hallmark 4: Unlimited replicative potential	31
Hallmark 5: Sustained angiogenesis	32
Mathematical models of pre-angiogenic growth	34

Mathematical models of capillary growth during angiogenesis	36
Hallmark 6: Invasion and metastasis	37
An enabling hallmark: Increased rates of mutation	38
2.5 Breast cancer	42
Structure of the breast	43
The role of estrogen and progesterone in the breast	43
Hormone replacement therapies and the development of cancer	45
2.6 Breast cancer cell lines - MCF-7 and T47D	46
2.7 Cell proliferation and the cell cycle	48
2.8 Proteins which encourage proliferation	50
2.9 Commitment to cell division and the the R point	52
How the R point is regulated	53
Inhibitors of cell cycle progression	54
3 Mathematical modelling of cell cycle progression	56
3.1 Flow Cytometry: detecting cell cycle phases	56
Flow cytometry and fluorescent DNA detection	57
Flow cytometry and detecting cell death	60
3.2 Previous mathematical models of cell cycle progression	62
Protein models of cell cycle progression	63
Age structured models	65
3.3 Our model of cell cycle progression	68
The inclusion of the storage phases	70

Steady-state: growth in unchanging environmental conditions	75
4 The MCF-7 breast cancer cell line	83
4.1 Determining the duration of the storage phases	83
Mitotic selection - an experimental procedure	83
A mitotic selection experiment using MCF-7 breast cancer cells	84
Approximating the duration of the storage phases using the mitotic selection experiment	87
4.2 Determining the remaining environment independent parameters	90
4.3 Model analysis	91
Average cell cycle duration and population doubling times	93
Sensitivity analysis: How the experiment independent variables influence the model	95
4.4 The doubling time and the average cell cycle duration	99
Other calculations of the average cell cycle duration from the literature	102
How the average duration in each of the phases is calculated in [28]	103
Discrete Simulation	105
Proof that by counting only some of the average residence times, we arrive at the formula presented in [28]	109
4.5 A novel representation of the model in equations (3.7)	111
Summary of our analysis of equations (3.7) after parameterisation to the MCF-7 cell line	117
5 Applying the parameterised model to experiments involving the MCF-7 breast cancer cell line	119

5.1	Applying our model to mitotically selected cells	119
	Modelling the pure cell population	121
	Modelling the impure cell population	121
	Results of optimisation	122
5.2	‘Slowly cycling population’?	127
	The experiment-independent variables, and their influence over model behaviour when not in steady-state	131
5.3	Changing Environmental Conditions	133
5.4	Biological literature and the γ function	135
5.5	Fitting the parameters from equation (5.2)	137
	Using experimentally determined cyclin D and myc	139
5.6	Applying the cell cycle model to other experiments involving growth factors	145
5.7	Modelling the effects of anti-estrogens	150
	Sensitivity analysis: solving the inverse problem for myc	155
5.8	Application of the model to tamoxifen exposed MCF-7 breast cancer cells	158
5.9	Tamoxifen exposure to mitotically selected cells	163
	The cytotoxic effects of tamoxifen	168
	Incorporation of cell death	169
	The form of $\beta_D(t)$ after continuous exposure to $12\mu\text{M}$ of tamoxifen	173
	Summary of our results of modelling cell cycle progression in MCF-7 breast cancer cells.	177

6 The T47D breast cancer cell line

179

6.1	A discussion of different experiments	180
6.2	A summary of experiments	181
	Results from [137]	181
	Results from [19]	182
	Results from [102]	182
	Results from [111]	182
	Results from [80]	182
	Summary of these experiments	183
	Correlation between experimental results	183
6.3	The storage phase durations for the T47D cell line	185
6.4	The γ function for the T47D cell line	190
	Generating the cyclin D profile from data in [81]	191
6.5	Applying the model to another cyclin D stimulation experiment	195
	Summary	198
7	Progesterin effects on cell cycle progression	201
7.1	Quantitative observations of protein changes in T47D breast cancer cells after exposure to progesterin	203
	Changes in the S phase proportion and protein concentrations after progesterin as observed in [84]	203
	Attempting to simulate S phase changes using known changes in cyclin D	207
	Changes in cyclin-CDK activity after progesterin exposure	209
7.2	Determining the molecular make-up of cyclin-CDK complexes using gel filtration chromatography	211

	Gel filtration chromatography and the elution profile	212
	Downsides of gel filtration chromatography	214
7.3	The elution profiles from [84] determined using gel filtration chromatography . .	215
	Interpreting the elution profiles from [84]	217
	The cyclin E1 profile	219
	The cyclin D profile	220
	The p27 profile	221
	The p21 profile	221
7.4	Quantitative analysis of the elution profiles from [84]	222
	The modelled elution profile for p27	222
	The modelled elution profile for cyclin E1	226
	The modelled elution profile for p21	229
	The modelled elution profile for cyclin D	231
	Summary	234
8	A model of progestin effects on cell cycle proteins	235
	Previous mathematical models of protein concentration changes during cell cycle progression	237
8.1	A simple model of cyclin E-CDK2 and p27	238
	Steady-states	241
	Stability analysis	241
8.2	An interesting relationship between cyclin E-CDK2 and p27	244
	Phosphorylation at T187	245

	Mechanism proposed by [106] in 1997	245
	Mechanism proposed by [74] in 1999	245
	Mechanism proposed by [140] in 1999	246
	Previous models of p27 and cyclin E in the literature	246
8.3	Including the mechanism of T187 phosphorylation in our model of p27 and cyclin E247	
	Nondimensionalisation	248
	Steady-state for the model in equation (8.6)	249
	Determining the rate parameters and starting conditions of our model	250
	Stability analysis	252
	Determining the drivers of our system, $D_{act}(t)$, $G_c(t)$ and $S(t)$	255
8.4	Modelling the experiment from [84]	257
	Model results and final parameterisation of β_{pe} and β_{ph}	258
	Steady-state analysis of our model	261
	The steady-state value of $P + P_E$ in terms of E	262
	The steady-state value of $E + P_E$ in terms of E	263
8.5	A discussion of experimental results from [37]	267
8.6	Expanding our model - introducing cyclin D	270
	Introducing the mechanism of reduction in cyclin D-CDK4 activity after progestin exposure	272
	Combining the model of cyclin D and p27 with the model of cyclin E-CDK2 and p27	274
	Determining the rate variables for the model in equation (8.18)	275

8.7	Combining the protein model from equations (8.18) with the cell cycle model from equations (3.7)	278
	Results of our combined model	279
	Summary of our model results	284
8.8	The effects of a second dose of progestin, administered at 40 hours after the first dose	285
	Simulating the second dose of progestin after 40 hours	286
	Summary	292
9	Conclusion	295
9.1	Thesis summary	295
9.2	Current avenues being explored for future work	297
9.3	Longer term views for future work	298
	Understanding the role of progesterone in the normal human breast	298
	Understanding breast cell proliferation during the normal menstrual cycle	299
	Gaining a better understanding of how therapies influence breast cancer risk . .	299
9.4	Final words	300

Declaration

I hereby declare this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Kate Simms 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.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. I also give permission for the digital version of my thesis to be made available on the web, via the Universitys digital research repository, the Library catalogue, the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Signature..... *Date*.....

Acknowledgments

My time as a PhD student has been one of the most rewarding periods of my life. I have had great supervisors and have met wonderful people throughout my PhD, whom I will never forget.

I would like to give special thanks to my supervisors Nigel Bean and Adrian Koerber for being patient and providing me with direction throughout my thesis. In particular, I am very grateful to my primary supervisor, Nigel, who always had time for me throughout my thesis and gave me encouragement and guidance when it was needed. I have said to many people that you are the best supervisor anyone can ever get!

I would also like to thank the team at the Hanson Institute, including my supervisor Wayne Tilley and Theresa Hickey, for being so welcoming and providing me with opportunities to communicate my results with a biological audience, and for answering my biological questions. In addition, I would also like to thank Professor Liz Musgrove from the Garvan Institute, for providing me with additional data and also for answering my biological questions.

Special thanks also goes to my fellow PhD students - I will thoroughly miss our long daily coffees and our long philosophical discussions! Also thanks to mum for listening to all my complaints so patiently. Lastly, a big thanks to Xiang, for being so encouraging and reading my work when I needed a second pair of eyes, and for just being there.

Abstract

The aim of this thesis is to develop mathematical models of cell cycle progression which can be used in conjunction with biological experiments. The thesis focusses on modelling processes which have biological relevance, and uses mathematics to investigate biological hypotheses about mechanisms which drive experimental results.

In this thesis, we introduce a mathematical model of cell cycle progression and apply it to the MCF-7 breast cancer cell line. The model considers the three typical cell cycle phases, which we further break up into model phases in order to capture certain features such as cells remaining in phases for a minimum amount of time. This results in a unique system of delay differential equations which are solved numerically using MATLAB. The model is also able to capture a uniquely important part of the cell cycle, during which time cells are responsive to their environment. The model parameters are carefully chosen using data from various sources in the biological literature. The model is then validated against a variety of experiments, and the excellent fit with experimental results allows for insight into the mechanisms that influence observed biological phenomena. In particular, the model is used to question the common assumption that a ‘slow cycling population’ is necessary to explain some results. A model analysis is also performed, and used to discuss misconceptions in the literature regarding the average length of the cell cycle. An extension is developed, where cell death is included in order to accurately model the effects of tamoxifen, a common first line anticancer drug in breast cancer patients. We conclude that the model has strong potential to be used as an aid in future experiments to gain further insight into cell cycle progression and cell death.

The model is then applied to the T47D cell line, which has significantly different cell cycle kinetics to the MCF-7 cell line. The aim of modelling this cell line, which is naturally receptive to the effects of progestins, is to model the effects of progestins on cell cycle progression. It is important to understand the effects of this substance, as it has been used in hormone replacement therapies, and its effects on cell cycle progression are still not understood.

In order to understand how progestins influence cell cycle progression, a more detailed protein model is developed to get a better understanding of how progestin influences protein concentrations within a cell. We find that progestin effects on cell cycle progression

are complex, and that progestin can be considered to be both a proliferative hormone and an anti-proliferative hormone, depending on the cell's previous history of progestin exposure, and on the length of time the cells have been exposed to progestin. The fact that the timing of progestin exposure can have different effects on cell behaviour has profound implications for treatments that contain progestins, such as combined hormone replacement therapies.

In summary, this thesis develops mathematical models representing different aspects of the cell cycle, and uses a variety of sources in the literature to parameterise the models. The model results are used to give insight into mechanisms that play a role in cell cycle progression under different experimental conditions. The models have the potential to be used alongside experiments, giving further insight into the mechanisms that influence events, such as cell cycle progression in the presence of hormones, as well as cell death.