A tumour suppressor role for FOXP3 and FOXP3-regulated microRNAs in breast cancer cells

NATASHA JACQUELINE MCINNES

Discipline of Paediatrics, School of Paediatrics & Reproductive Health, University of Adelaide

September 2012

A thesis submitted to the University of Adelaide as the requirement for the degree of Doctor of Philosophy

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Thesis Corrigendum

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This corrigendum has been included in this thesis due to errors identified in the analysis of Quantitative Real-Time PCR experiments that were performed after this thesis was submitted. These errors were typographical, and arose in the manual transfer of numbers from raw data files (Rotorgene 6000 software) to data analysis files (Microsoft Excel software). It is therefore possible that errors of a similar nature arose during the preparation of figures for this thesis.

Importantly, it should be noted that despite the potential minor errors present in the Quantitative Real-Time PCR data presented in this thesis, this was not the only method used to investigate the hypotheses. Additional experiments that support the results of the Quantitative Real-Time PCR experiments include western blots, luciferase assays and growth assays. It is therefore extremely unlikely that the presence of minor errors in the Quantitative Real-Time PCR analyses would influence the overall significance and conclusions of this thesis.

Unfortunately, due to misplacement of the data files used to generate the figures in this thesis, it is not possible to provide replacement figures for the Quantitative Real-Time PCR experiments performed. However, one experiment relating to the work performed in Chapter 4 of this thesis was performed after thesis submission, and supports Figure 4.6a of this thesis. The results of this additional experiment and a comparison with the results shown in Figure 4.6a are discussed in further detail on this disc.

Natasha Jacqueline McInnes

Date: 3/11/14

corbett RESEARCH
A Division of Corbett Life Science

14 Hilly Street Mortlake NSW 2137 Australia
T +61 2 9736 1320
F +61 2 9736 1364
W www.corbettlifescience.com

Quantitation Report

Experiment Information

Run Name	RPL13a SATB1 1.3.13
Run Start	1/03/2013 11:56:39 AM
Run Finish	1/03/2013 12:58:59 PM
Operator	natasha
Notes	BT 231 PNA and pre-miR 155: SATB1 and RPL13a
Run On Software Version	Rotor-Gene 4.4.1
Run Signature	The Run Signature is valid.
Gain Green	5.

Quantitation Information

Threshold	0.01832
Left Threshold	1.000
Standard Curve Imported	No
Standard Curve (1)	N/A
Standard Curve (2)	N/A
Start normalising from cycle	1
Noise Slope Correction	No
No Template Control Threshold	0%
Reaction Efficiency Threshold	Disabled
Normalisation Method	Dynamic Tube Normalisation
Digital Filter	Light
Sample Page	Page 1
Imported Analysis Settings	

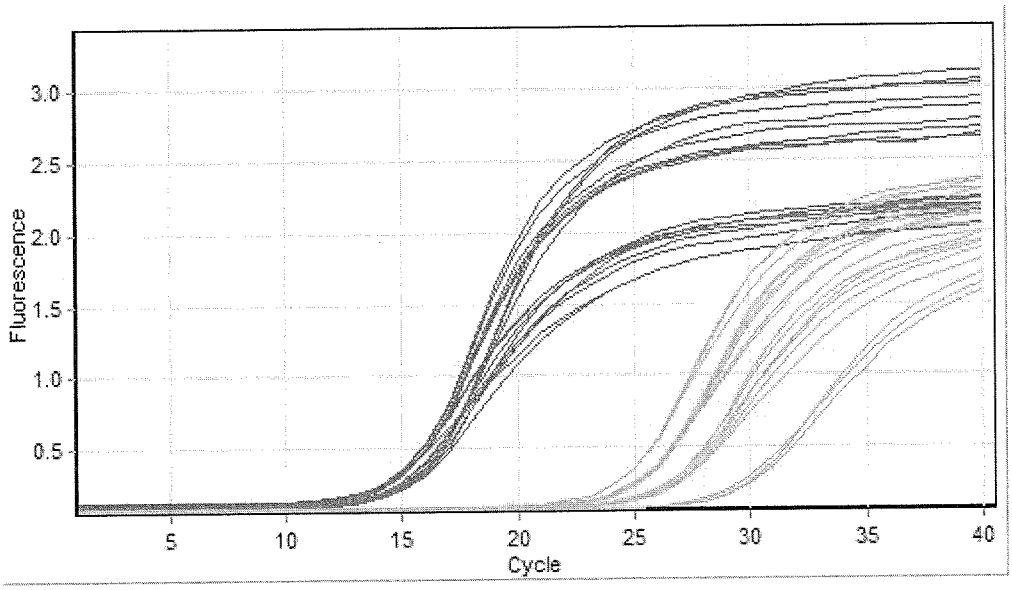
Messages

Message

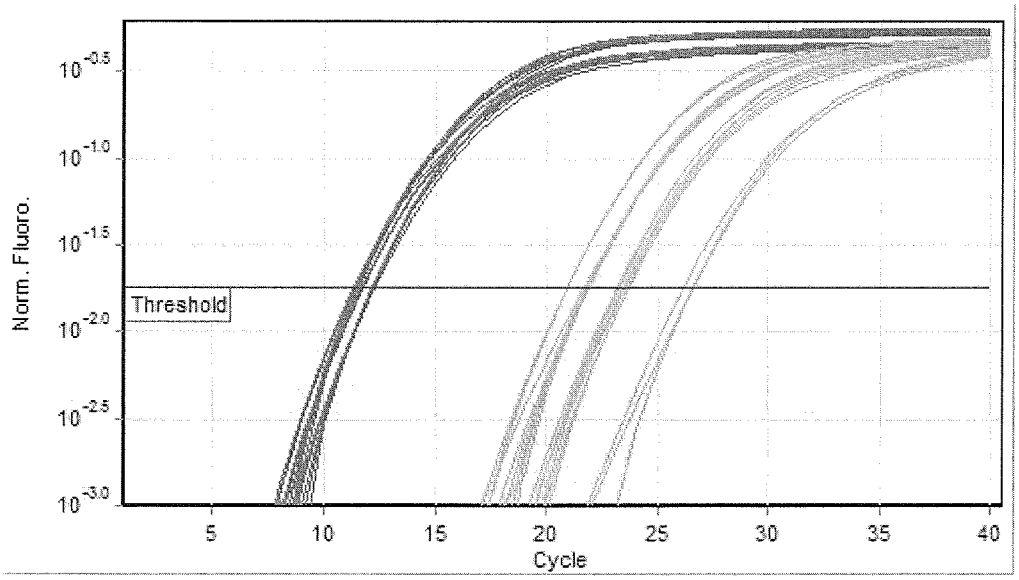
Profile

Cycle	Cycle Point
Hold @ 95°c, 3 min 0 secs	
Cycling (40 repeats)	Step 1 @ 95°c, hold 3 secs
	Step 2 @ 60°c, hold 25 secs, acquiring to Cycling A([Green][1][1])
Melt (50-99°c) , hold secs on the 1st step, hold 5 secs on next steps, Melt A([Green][1][1])	

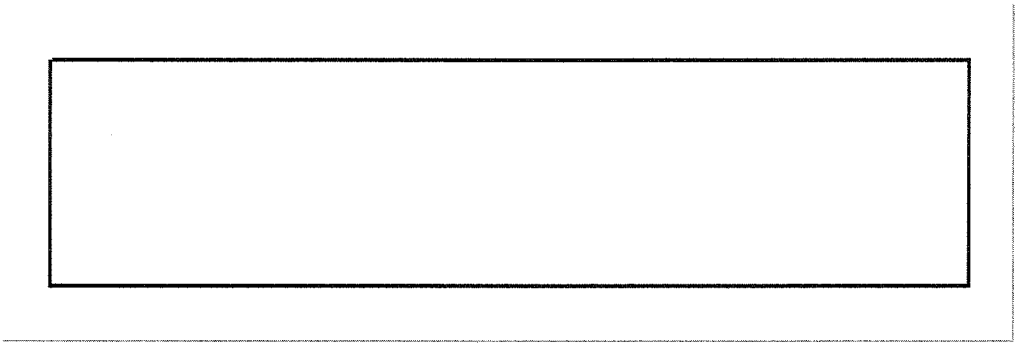
Raw Data For Cycling A.Green



Quantitation data for Cycling A.Green



Standard Curve



No.	Colour	Name	Type	Ct	Rep. Ct	Rep. Ct Std. Dev.	Rep. Ct (95% CI)
34		Bt alone RPL	Unknown	12.24	12.21	0.02	[12.16 , 12.27]
35		Bt alone RPL	Unknown	12.19			
36		Bt alone RPL	Unknown	12.22			
37		Bt+miR NC RPL	Unknown	11.51	11.52	0.08	[11.32 , 11.72]
38		Bt+miR NC RPL	Unknown	11.45			
39		Bt+miR NC RPL	Unknown	11.60			
40		Bt+miR-155 RPL	Unknown	11.58	11.57	0.03	[11.50 , 11.63]
41		Bt+miR-155 RPL	Unknown	11.58			

(Continued on next page)...

No.	Colour	Name	Type	Ct	Rep. Ct	Rep. Ct Std. Dev.	Rep. Ct (95% CI)
42		Bt+miR-155 RPL	Unknown	11.54			
49		231 alone RPL	Unknown	11.79	11.75	0.05	[11.64 , 11.86]
50		231 alone RPL	Unknown	11.76			
51		231 alone RPL	Unknown	11.70			

52		231+miR NC RPL	Unknown	11.36	11.37	0.01	[11.35 , 11.39]
53		231+miR NC RPL	Unknown	11.37			
54		231+miR NC RPL	Unknown	11.37			
55		231+miR-155 RPL	Unknown	12.23	12.25	0.07	[12.09 , 12.42]
56		231+miR-155 RPL	Unknown	12.33			
57		231+miR-155 RPL	Unknown	12.20			
64		Bt alone SAT	Unknown	21.73	21.82	0.09	[21.60 , 22.05]
65		Bt alone SAT	Unknown	21.92			
66		Bt alone SAT	Unknown	21.82			
67		Bt+miR NC SAT	Unknown	20.97	20.96	0.00	[20.95 , 20.97]
68		Bt+miR NC SAT	Unknown	20.96			
69		Bt+miR NC SAT	Unknown	20.96			
70		Bt+miR-155 SAT	Unknown	21.82	21.84	0.04	[21.74 , 21.95]
71		Bt+miR-155 SAT	Unknown	21.89			
72		Bt+miR-155 SAT	Unknown	21.82			
79		231 alone SAT	Unknown	23.51	23.60	0.08	[23.40 , 23.80]
80		231 alone SAT	Unknown	23.64			
81		231 alone SAT	Unknown	23.66			
82		231+miR NC SAT	Unknown	23.16	23.26	0.11	[22.98 , 23.53]
83		231+miR NC SAT	Unknown	23.24			
84		231+miR NC SAT	Unknown	23.38			
85		231+miR-155 SAT	Unknown	26.60	26.45	0.26	[25.81 , 27.08]
86		231+miR-155 SAT	Unknown	26.59			
87		231+miR-155 SAT	Unknown	26.15			

Warning: The following samples were not analysed :
43-Bt+PNA NC RPL 44-Bt+PNA NC RPL 45-Bt+PNA NC RPL 46-Bt+PNA-155 RPL 47-Bt+PNA-155 RPL 48-Bt+PNA-155 RPL 58-231+PNA NC RPL 59-231+PNA NC RPL 60-231+PNA NC RPL 61-231+PNA-155 RPL 62-231+PNA-155 RPL 63-231+PNA-155 RPL 73-Bt+PNA NC SAT 74-Bt+PNA NC SAT 75-Bt+PNA NC SAT 76-Bt+PNA-155 SAT 77-Bt+PNA-155 SAT 78-Bt+PNA-155 SAT 88-231+PNA NC SAT 89-231+PNA NC SAT 90-231+PNA NC SAT 91-231+PNA-155 SAT 92-231+PNA-155 SAT 93-231+PNA-155 SAT

Legend:
NEG (NTC) - Sample cancelled due to NTC Threshold.
NEG (R. Eff) - Sample cancelled as efficiency less than reaction efficiency threshold.

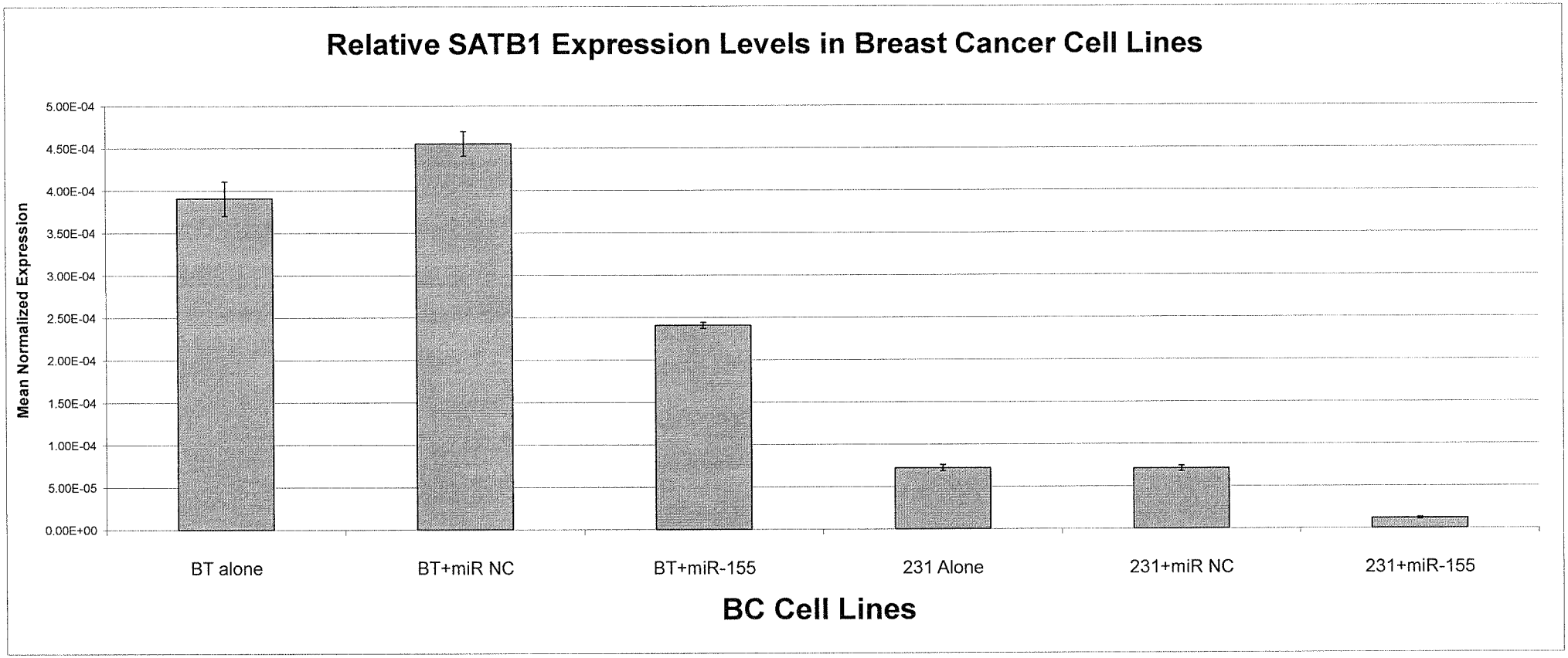
SATB1 (BT549 & MDA-MB-231)

Calculation Procedure for MNE	1
Threshold for SEM in %	20.0

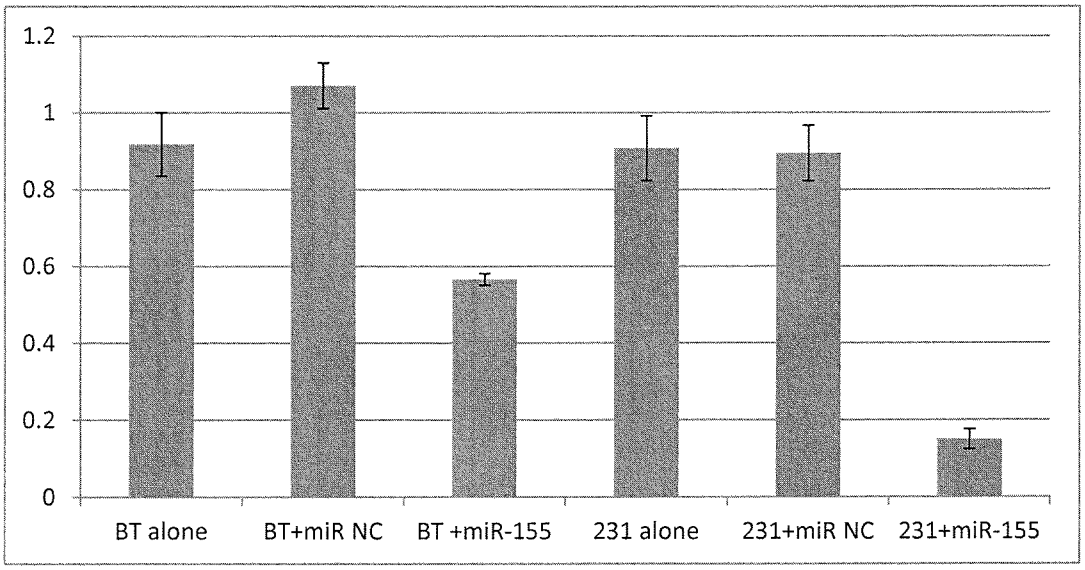
Well	Description	CT of Target Gene	CT of Reference Gene	Normalized Expression
A1	BT alone	21.73	12.24	4.25E-04
A2		21.92	12.19	3.55E-04
A3		21.82	12.22	3.92E-04
A4	BT+miR NC	20.97	11.51	4.49E-04
A5		20.96	11.45	4.34E-04
A6		20.96	11.60	4.83E-04
A7	BT+miR-155	21.82	11.58	2.47E-04
A8		21.89	11.58	2.35E-04
A9		21.82	11.54	2.40E-04
A10	231 Alone	23.51	11.79	7.94E-05
A11		23.64	11.76	7.04E-05
A12		23.66	11.70	6.64E-05
B1		23.16	11.36	7.62E-05
B2	231+miR NC	23.24	11.37	7.22E-05
B3		23.38	11.37	6.49E-05
B4		26.60	12.23	1.04E-05
B5	231+miR-155	26.59	12.33	1.12E-05
B6		26.15	12.20	1.43E-05
B7				
B8				
B9				
B10				
B11				
B12				
C1				
C2				
C3				
C4				
C5				
C6				
C7				
C8				
C9				
C10				
C11				
C12				
D1				
D2				
D3				
D4				
D5				
D6				
D7				
D8				
D9				
D10				
D11				

Data Analysis

Mean of Triplicates			
Description	Mean Normalized Expression	SE of Mean Normalized Expression	SE of Mean Normalized Expression in %
BT alone	3.91E-04	2.03E-05	5.19
BT+miR NC	4.55E-04	1.45E-05	3.19
BT+miR-155	2.41E-04	3.71E-06	1.54
231 Alone	7.21E-05	3.85E-06	5.34
231+miR NC	7.11E-05	3.30E-06	4.65
231+miR-155	1.20E-05	1.19E-06	9.96

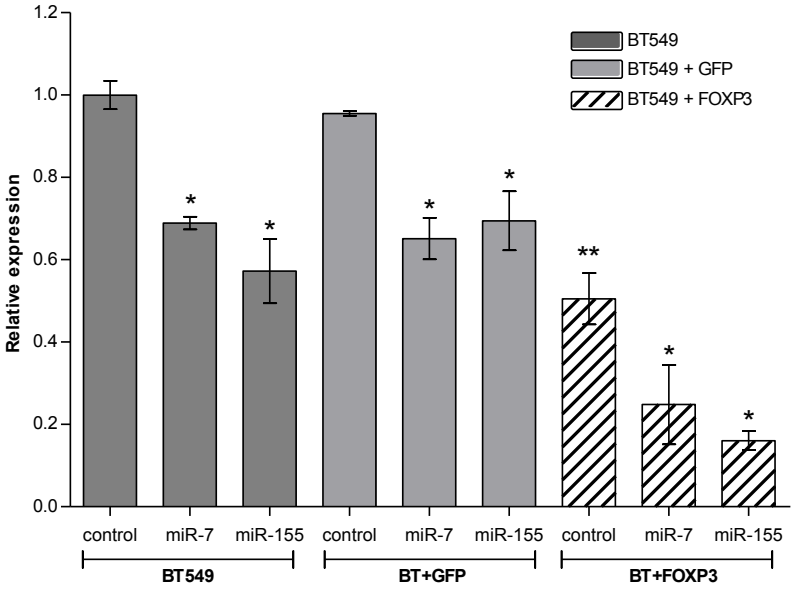

Relative SATB1 Expression Levels in Breast Cancer Cell Lines
Mean Normalized Expression
5.00E-04
4.50E-04
4.00E-04
3.50E-04
3.00E-04
2.50E-04
2.00E-04
1.50E-04
1.00E-04
5.00E-05
0.00E+00
BT alone
BT+miR NC
BT+miR-155
231 Alone
231+miR NC
231+miR-155
BC Cell Lines

Cell line/treatment	Normalised expression	Relative expression	Average	Standard Deviation
	0.000425385	1		
	0.000355122	0.834825725		
BT alone	0.000391554	0.920468984	0.918432	0.082605984
	0.000449382	1.056413368		
	0.000433737	1.019635089		
BT+miR NC	0.000483021	1.135491629	1.070513	0.059201282
	0.000247384	0.581552229		
	0.000234544	0.55136967		
BT+miR-155	0.000240385	0.565099532	0.566007	0.015111735
	7.94326E-05	1		
	7.04151E-05	0.886476064		
231 alone	6.64289E-05	0.836292587	0.90759	0.083871114
	7.61627E-05	0.958834507		
	7.21782E-05	0.908672108		
231+miR NC	6.48805E-05	0.816799557	0.894769	0.072030964
	1.03604E-05	0.130430006		
	1.12161E-05	0.141202864		
231+miR-155	1.42831E-05	0.17981439	0.150482	0.025967036

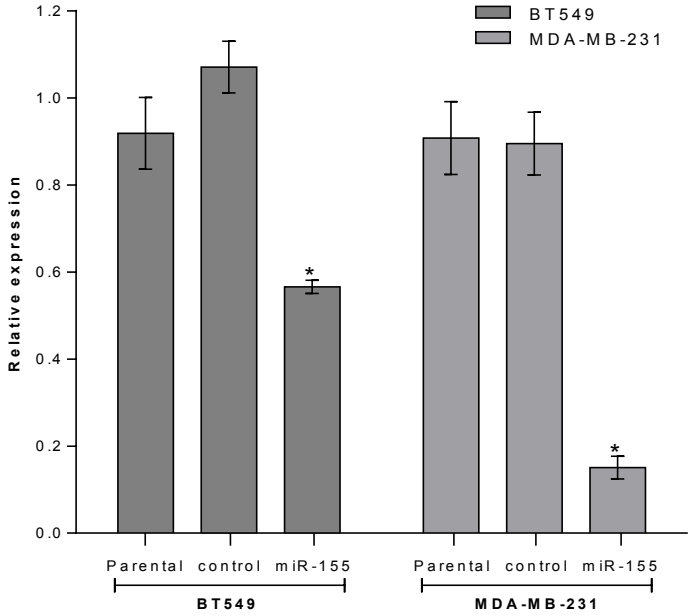


Corrigendum Figure 1 ***Endogenous SATB1 expression is reduced by microRNAs***

a)



b)



a) Figure 4.6a as it appears in this thesis. Expression of endogenous *SATB1* mRNA is reduced when pre-miR-7 or pre-miR-155 is transiently expressed in BT549 cells. *SATB1* levels in the parental cells (dark bars) and GFP-transduced control lines (grey bars) are reduced by the transfection of miR-7 or miR-155. Overexpression of *FOXP3* alone (hatched bars) reduces *SATB1* levels, compared with the control cell lines. Transient transfection of either pre-miR further reduces *SATB1* in these cells. (Triplicate RNA analysis of n=3 transfection pools, *$p<3.12x10^{-5}$, **$p=1.03x10^{-12}$. b) Figure derived from an experiment performed after this thesis was submitted. Expression of endogenous *SATB1* mRNA is reduced when pre-miR-155 is transiently expressed in BT549 (dark bars) and MDA-MB-231 (grey bars) breast cancer cell lines. Transient transfection of parental cells with a pre-miR control does not reduce *SATB1* levels, while transient transfection with pre-miR-155 result in significantly reduced *SATB1* levels. (Triplicate RNA analysis of n=1 transfection pool, *$p<0.0002$).

Corrigendum Discussion

In this thesis, the quantitative RT-PCR analysis shown in Figure 4.6a (and in Corrigendum Figure 1a) demonstrates that transient transfection of microRNA precursors pre-miR-7 and pre-miR-155 into the BT549 cell lines resulted in a significant reduction in the *SATB1* mRNA levels when compared with the control pre-miR-transfected lines (35 to 38% reduction, $p=6.35x10^{-5}$, and 35 to 45% reduction, $p=1.4x10^{-9}$ respectively). These results were supported by luciferase assays, which demonstrated direct binding of the microRNAs to the SATB1 3' UTR, and by western blot analyses, which demonstrated that transient transfection of the BT549 cell lines with pre-miR-7 or pre-miR-155 resulted in a reduction in SATB1 protein levels. It was therefore concluded that these FOXP3-regulated microRNAs target endogenous *SATB1*, thus supporting the hypothesis of Chapter 4: "FOXP3 is able to regulate the expression of *SATB1* by binding to the promoter region of *SATB1* and also by regulating miRs that bind to the 3'UTR of *SATB1*".

A similar quantitative RT-PCR experiment was performed after completion of this thesis, in which the BT549 and MDA-MB-231 breast cancer cell lines were both transiently transfected with pre-miR-155 (Corrigendum Figure 1b). As found in Figure 4.6a of this thesis, transient transfection of the BT549 cell line with pre-miR-155 results in a significant reduction (~45%) of endogenous *SATB1* message levels when compared with the un-transfected parental and control pre-miR-transfected lines. This miR-155-dependent downregulation of SATB1 is also seen in the MDA-MB-231 cell line (~85% reduction in *SATB1* message levels), once again supporting the hypothesis of this thesis that FOXP3-regulated microRNAs target *SATB1*.

The raw Corbett Rotor-Gene data for this experiment can be found on this disc in the document entitled "NJM_corrigendum_Raw data.pdf". The Microsoft Excel analysis of the data is also located on this disc in the document "NJM_corrigendum_Analysed data.pdf", as are the calculations performed in Microsoft Excel in which the *SATB1* levels were determined relative to the parental controls ("NJM_corrigendum_Analysed data_relative.pdf"). Together, these documents show that no errors have occurred in the transfer of numbers from the original raw data to the analysed data for Corrigendum Figure 1b.

Overall, it is clear that despite the possibility that there are minor errors in the analysis of the quantitative RT-PCR experiments performed for this thesis, significant evidence from other experiments performed both during and after completion of this thesis suggest that the original hypotheses and conclusions of this thesis are valid.

Table of Figures & Tables

Abstract

During their lifetime, 1 in 9 Australian women will be diagnosed with breast cancer, a disease that arises due to mutations and epigenetic modifications to tumour suppressor genes and cancer-promoting oncogenes. This thesis investigates the tumour suppressive role of a transcription factor called Forkhead box Protein 3 (FOXP3) in breast cancer. Little is known regarding its role in the breast and therefore identification of FOXP3-sensitive pathways has the potential to highlight novel targets for breast cancer diagnosis and therapy.

FOXP3 is a 'master regulator' in immunosuppressive T regulatory cells, where it is essential for both cell development and function. It was previously thought that *FOXP3* expression was restricted to these immune cells, however recent studies have identified *FOXP3* expression in breast epithelia, where it has potential tumour suppressor properties. *FOXP3* is mutated or has reduced expression in a significant proportion of human breast cancer samples, and loss of *FOXP3* has been linked to increased mammary tumour formation in animal models. Few targets of FOXP3 in the breast have been identified, but it is known to directly repress the *HER2* and *SKP2* oncogenes while maintaining expression of the *p21* tumour suppressor gene.

A number of groups have shown that in T regulatory cells, FOXP3 regulates a number of small, non-coding RNAs called microRNAs (miRs). Importantly, many studies have reported extensive microRNA deregulation in human diseases, including breast cancer, and

it was therefore hypothesised that similar regulation of miRs by FOXP3 occurs in breast epithelia.

This thesis describes how FOXP3 induces two microRNAs, miR-7 and miR-155, in breast epithelial cells, with these miRs contributing to FOXP3-mediated tumour suppressive activity. One way this is achieved is through co-operation with FOXP3 in a feed-forward regulatory loop to suppress an oncogene called *SATB1*. *SATB1* is highly overexpressed in late-stage breast cancers and promotes metastasis, the final and most fatal stage of breast cancer. This work has established that the *SATB1* promoter is a direct target for FOXP3 repression and that miR-7 and miR-155 target the 3'UTR of *SATB1* for further suppression. Re-introduction of *FOXP3* into breast cancer cells using lentiviral technology results in reduced cell proliferation and invasion potential, supporting a role for FOXP3 as a tumour suppressor.

To further understand the physiological importance of *FOXP3* loss in cancer development, this work also investigated the role of FOXP3 in normal and immortalised breast epithelial cells, with results suggesting that *FOXP3* expression prevents the acquisition of a cancerous phenotype. One way that it may achieve this is by maintaining elevated levels of miR-7 and miR-155. After further investigation, it was found that FOXP3 and miR-7 both have the potential to reduce epidermal growth factor receptor signalling and reduce resistance to apoptosis.

In summary, this work describes a role for FOXP3 and the FOXP3-regulated microRNAs miR-7 and miR-155 in preventing the transformation of healthy breast epithelium to a

cancerous phenotype. One way this is achieved is through a novel feed-forward mechanism by which FOXP3 and FOXP3-regulated miRs work together to suppress the pro-metastatic oncogene *SATB1*. This thesis provides important insight into the tumour suppressive role of FOXP3 in breast epithelia and with further investigation, this new knowledge may form the basis for the development of a novel and effective targeted breast cancer therapeutic.

Declaration

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 Natasha Jacqueline McInnes 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.

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* McInnes, N *et al.* (2012) *FOXP3 and FOXP3-regulated microRNAs suppress SATB1 in breast cancer cells,* Oncogene 23; 31(8): 1045-1054

Natasha Jacqueline McInnes Date:

Publications, Presentations and Awards

Publications:

McInnes N, Sadlon TJ, Brown CY, Pederson S, Beyer M, Schultze JL, McColl S, Goodall GJ, Barry SC (Feb 2012), FOXP3 and FOXP3-regulated microRNAs suppress SATB1 in breast cancer cells. *Oncogene.* (Full article available in the Appendix)

Hill D, Eastaff-Leung N, Bresatz-Atkins S, Warner N, Ruitenberg J, Krumbiegel D, Pederson S, **McInnes N**, Brown CY, Sadlon T, Barry SC, Inhibition of activation induced CD154 expression: A rapid assay of human regulatory T cell suppressor function. *Immunology and Cell Biology.*

Awards Received:

- Young Investigator Award (2012)
- Student Poster Prize: Robinson Institute Research Symposium (2012)
- Supplementary Scholarship: School of Paediatrics and Reproductive Health (2012)
- Student Poster Prize: CRC for Biomarker Translation Student Retreat (2011)
- WCHRI young investigator (2010/2011)
- Australian Postgraduate Award (2009-2012)
- CRC for Biomarker Translation Top-up Scholarship (2009-2012)
- Best student presentation: CRC for Biomarker Translation Annual Retreat 2009

Abstracts Published:

McInnes N, Sadlon TJ, Pederson S, McColl S & Barry SC. FOXP3 and FOXP3-regulated microRNAs suppress SATB1 in breast cancer cells (2012) SA Breast Cancer Research Show

McInnes N, Sadlon TJ, Pederson S, McColl S & Barry SC. FOXP3 and FOXP3-regulated microRNAs suppress SATB1 in breast cancer cells (2011) CRC for Biomarker Translation Student Retreat.

McInnes N, Sadlon TJ, Pederson S, McColl S & Barry SC. FOXP3 and FOXP3-regulated microRNAs suppress SATB1 in breast cancer cells (2011) University of Adelaide Faculty of Health Sciences Postgraduate Conference.

McInnes N, Sadlon TJ, Brown CY, Pederson S, Kochetkova M, McColl S & Barry SC. The molecular basis of FOXP3-mediated tumour suppression in breast epithelium (2010) Miami 2010 Winter Symposium: Targeting Cancer Invasion and Metastasis.

McInnes N, Sadlon TJ, Pederson S, McColl S & Barry SC. FOXP3 and FOXP3 regulated microRNAs suppress SATB1 expression in breast cancer cell lines (2010) CRC for Biomarker Translation Annual Retreat.

McInnes N. The molecular basis of FOXP3-mediated tumour suppression in breast epithelium (2009) CRC for Biomarker Translation Annual Retreat.

Grants awarded:

NHMRC (2011): $536,010.00

WCH Foundation Research Project Grant (2011): $50,000.00

Acknowledgements

First and foremost I would like to thank my supervisors, Simon, Tim and Shaun. Thank you for agreeing to keep me after honours, and for happily accepting the fact that I was going to be hanging around you for at least another few years. Simon, your enthusiasm about science is an inspiration, and you always manage to make me see the positive side of things, even when I'm at my lowest. Thank you for listening to all of my problems, for buying me the occasional coffee and for providing some serious competition at Christmas party activities. One day I hope that my passion for science will rival yours- thank you for agreeing to have me as your student and for putting me on the path to a promising future!

Tim, thank you for being an incredible walking and talking encyclopaedia! I'm always astounded by just how much you know about everything to do with science, and you're assistance with understanding why an experiment has failed has made me grow as a scientist. I've noticed over the last few years that I've really grown to understand so much more about research and a lot of that growth I can attribute to you. Thanks for all the laughs (and for the occasional use of your Cathedral Hotel discount card), you've made my PhD experience a pleasant one.

To Shaun McColl, thank you for your assistance with experimental design and for your support. To our German collaborators, Marc Beyer and Joachim Schultz, thank you for your advice and for providing the vectors and reagents I needed to perform my experiments. Thank you also to the members of the Goodall group, for your expertise in the area of microRNAs and for providing reagents at the beginning of my PhD.

To all the past and present members of the lab, thank you for being absolutely incredible, supportive, fun and inspirational people. Steve, Suzanne, John, Danika, Liz, Cheryl, Nicola, Sylvie, Sonia, Old Tessa, New Tessa, Ying, Grace, Kristen, Kelly and Chloe, I hope that each of you know just how important I think you all are. I spend more time in the lab than I do anywhere else, so I'm lucky to be surrounded by such a great group of people. Thank you for the coffee breaks, the advice, for putting up with my excel-related tantrums, and for just being my friends. Sylvie, thanks for running all of my Flow and FACS tubes, you're an extremely patient woman! A special thanks to my fellow PhD students Steve and John, for understanding what it's like - I'm so glad I didn't have to go through this process alone! Also Steve, a big thank you for making time in your busy schedule to help me out with stats; you truly are a superhero Statman! Thanks also to Suzanne (aka Suanie, aka Lady FlowJo), for the countless lunch dates, for reading my chapters, for providing emotional support and for your help using the Canto- even though those experiments didn't work, you did a mighty fine job of showing me how to use the machine.

To all of my friends outside of science, thank you all so much for your support, the encouraging phone calls and text messages, and for still being my friends when I abandoned you all to write my thesis. I can't wait to spend a bit more time with you all once this is over. Special thanks goes to Bree for always checking up on me and for organising our "de-stress" gym, dinner and DVD nights, you know I think you're awesome.

To my family (including my new Sinclair/Huxtable family): thank you for believing in me, for encouraging me, for being interested in what I'm doing, for the financial support and for the countless meals. You are all wonderful, and I don't think I could have made it through without your support. Mum and Dad, thank you for giving me a wonderful upbringing, for spending so much money on my education and for being there whenever I needed you. I hope you realise how lucky I am to have the two of you. Monica- thanks for being such a good big sister and for all of the supportive phone calls (and the chocolate parcels!)- Even though you're in Melbourne it's been great venting to you. Alan, you're an awesome brother. Even though you don't really know what I'm doing, you provide a nice distraction from study! Thanks for knowing how to make me laugh.

Finally, but very importantly, thank you to my husband Thomas. You are an incredible person, my best friend and an amazing husband. You have been unbelievably supportive and I want to thank you for holding your own career back so that you could support mine. It's been a difficult 3½ years, but the highlight of my PhD years was definitely getting married to you. And don't worry, now that this is over I promise to finally get a 'real job'!

Abbreviations

ºC	degrees Celsius
Δ3	*FOXP3* isoform lacking exon 3
3'UTR	3 prime untranslated region
µg	microgram
µL	microLitre
µM	microMolar
Ab	Antibody
APC	Antigen Presenting Cells
ATF-2	Activating Transcription Factor 2
BC	breast cancer
BME	Basement Membrane Extract
bp	base pairs
BR	binding region
Breg	Regulatory B cell
BSA	Bovine Serum Albumin
cDNA	complimentary DNA
ChIP-on-chip	combination of Chromatin Immuno-Precipitation and microarray technology
cPPT	central polypurine tract
CSC	cancer stem cell
Ct	cycle threshold
dH_20	distilled water
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Dinucleotide triphosphate
EDTA	Ethylene diamine tetra-acetic acid
EGFR	Epidermal Growth Factor Receptor

EMT	Epithelial-to-mesenchymal transition
ER	Estrogen Receptor
ERBB2/HER2	Human Epidermal growth Factor Receptor 2
FACS	Fluorescence activated cell sorter
FCS	Foetal Calf Serum
FL	full length
FOXP3	Forkhead box Protein 3
FKH	Forkhead
g	gravitational force
gDNA	genomic DNA
GFP	Green Fluorescent Protein
HAT	Histone Acetyl-Transferase
HDAC	Histone deacetylase
HER2	Human Epidermal growth factor Receptor 2
HMEC	Human Mammary Epithelial cell line
HoxD10	Homeobox D10
IDC	Invasive ductal carcinoma
IDC-NOS	Invasive ductal carcinoma not otherwise specified
IFN	Interferon
Ig	Immunoglobulin
ILC	Invasive lobular carcinoma
IPEX	Immune dysregulation Polyendocrinopathy Enteropathy X-linked disease
IRES	Internal Ribosome Entry Site
IRS	insulin receptor substrate
iTreg	inducible T regulatory cell
Kb	Kilobase
KO	knockout
L	Litre
LB	Luria broth
LPS	Lipopolysaccharide

LV	lentivirus
LZ	Leucine zipper
M	Molar
MaSC	mammary gland stem cell
MCS	multiple cloning site
MDSC	Myeloid-derived suppressor cell
mg	milligram
miR	microRNA
mL	millilitre
MOI	multiplicity of infection
mRNA	messenger ribonucleic acid
MRP	multidrug resistance-associated protein
mTor	mammalian target of rapamycin
MW	molecular weight
n	sample size
NaCl	sodium chloride
NC	nitrocellulose
NES	nuclear export sequences
NFAT	Nuclear Factor of Activated T cells
NFκB	Nuclear factor kappa B
NLS	nuclear localisation sequences
nM	nanoMolar
ns	not significant
nTreg	natural T regulatory cell
o/n	overnight
PAK-1	p21-activated kinase
PARP-1	Poly ADP-ribose Polymerase 1
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PR	progesterone receptor

PRE	post-transcriptional regulatory element
P/S	Penicillin/Streptomycin
RISC	RNA-induced silencing complex
ROR	Retinoic acid receptor-related orphan receptor
rpm	revolutions per minute
RPMI	Roswell Park Memorial Institute medium
RRE	Rev-responsive element
RSV	Rous sarcoma virus promoter
RT	room temperature
RT-PCR	Reverse transcription real time polymerase chain reaction
SATB1	Special AT-rich binding protein 1
SD	standard deviation
SEM	Standard error of the mean
shRNA	short hairpin RNA
siRNA	small interfering RNA
SKP2	S-phase Kinase Protein 2
STAT3	Signal Transducer and Activator of Transcription 3
TCF4	transcription factor 4
TDLU	Terminal Ductal Lobular Units
TGFβ	Tumour growth factor β
Th	T helper cell
TIP	Tat-interactive protein
TLDA	Taqman Low Density Array
TN	Triple negative
Treg	T regulatory cell
TSA	Trichostatin A
VEGF	Vascular Endothelial Growth Factor
WT	wildtype
ZF	Zinc finger