MOLECULAR CHARACTERISATION, REGULATION AND EVOLUTIONARY ANALYSIS OF *UROPLAKIN 1B*: A TETRASPANIN FAMILY MEMBER

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A thesis submitted for the degree of
Doctor of Philosophy (PhD)

Presented to the Department of Surgery, The
Queen Elizabeth Hospital, in the Faculty of Health Sciences,
The University of Adelaide, South Australia

-June, 2003-

The important thing is not to stop questioning. Curiosity has its own reason for existing. One cannot help but be in awe when he contemplates the mysteries of eternity, of life, of the marvellous structure of reality.

Albert Einstein.

Every moment of your life is infinitely creative and the universe is endlessly bountiful.

Mohandas Karamchand Gandhi.

Curiosity is, in great and generous minds, the first passion and the last.

Samuel Johnson.

September 15th, 2003

Errata

Chapter 1
Data is used in the singular, instead of datum.

Chapter 2 (Page 58)
Sterile MilliQ – spelling correction.

Chapter 3 and Appendix A
Homo sapien is used, instead of *Homo sapiens*.

Chapter 5 (Pages 142B, 143, 143B, 146D and 146E)
Bisulfite should be used, instead of bisulphite.

Figure 5.14 (Page 147A)
TE-N clones in the table should be 1 through 12. The last two clones were accidentally both named 10.

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Summary

Uroplakin 1B (UPKIB) is an integral structural protein interacting with uroplakins 1A, 2 and 3 to form hexameric plaques along the bladder lumen in the asymmetric unit membrane of urothelial umbrella cells in humans and other mammals. UPKIB mRNA expression is deregulated in transitional cell carcinomas (TCCs), however the mechanisms of regulation of *UPKIB* have not been established.

Using genome databases, a *Xenopus* UPKIB homologue was identified. Maximum Parsimony and BAMBE (Bayesian Analysis in Molecular Biology and Evolution) data support a close evolutionary relationship between mammalian and amphibian UPKIB mRNA. Using Unigene, UPKIB human expressed sequence tags were identified in tissues including brain, skeletal muscle and liver, suggesting the relatively widespread distribution of this membrane protein. The UPKIB genomic structure was also deduced using genome databases. Contig AC083800, identified in a high throughput genomic sequence database, spanned *UPKIB* and 9 exons and 8 introns were defined.

A 67bp 5' untranslated region was identified using 5' rapid amplification of cDNA ends. This product was sequenced and a putative UPKIB promoter and transcription start site was deduced. Contig AC083800 spanned the transcription start site and putative promoter. Transcription factor binding motif prediction programs detected no TATA box, but did predict a CCAAT box and several binding motifs including 4 Sp-1 sites and a NF_KB site. A weak CpG island was identified within a 0.5kb region including the putative promoter, exon 1 and intron 1, which was 54% GC rich with CpG:GpC ratio of 0.46, containing 15 CpG dinucleotides.

Seven TCC cell lines and five peripheral blood lymphocyte samples were analysed for UPKIB expression using RT-PCR and two cell lines expressed UPKIB transcripts. Eleven CpG sites in the putative promoter were investigated for methylation using bisulfite modification analysis in normal PBL, TCC cell lines and patient TCC samples. An inverse correlation was established in TCC cell lines between UPKIB mRNA expression and degree of methylation. 5-Aza-2'deoxycytidine induced UPKIB mRNA expression in T24 cells, previously observed not to express UPKIB. Sequence analysis of patient samples revealed more complex CpG methylation patterns, reflecting tumour heterogeneity. In summary, the uroplakin 1B gene has been characterised and one mechanism of regulation of gene expression involves methylation.

Declaration of Originality

The work in this thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution 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 of the thesis.

I give consent to this copy of my thesis, when deposited in the University of Adelaide Barr Smith library, being available for photocopying and loan when accepted for the award of the degree.

Andrea Varga
21st of June, 2003

Acknowledgements

With deep gratitude I acknowledge the support, guidance and patience of my Supervisor Dr Prue Cowled in the Department of Surgery during the course of this degree. Dr Cowled's advice on my experimental work and her constructive comments during the writing process were much appreciated. I would also acknowledge the assistance and support of Professor Guy Maddern, Dr Claudia Fazeli, Ms Sue Millard, Mrs Lefta Leonardos and others within the department.

With deep gratitude I thank Dr Graham Webb from the University of Adelaide Departments of Obstetrics and Gynaecology and Animal Sciences for his continuing support and mentorship over the past few years. It was through his thoughtfulness and assistance that the evolutionary chapter was initiated. Dr Webb introduced me to Dr Hugh Campbell from the Department of Evolutionary and Molecular Genetics at the Research School of Biological Sciences, ANU, Canberra, with whom I had the pleasure of studying evolutionary biology and hydrophilicity profiles. With many thanks, I also acknowledge the continued support of Dr Rory Hope, Mr Scott Spargo and Mr David Wheeler from the Department of Molecular Biosciences at the University of Adelaide for their assistance with Chapter 3 and with Maximum Parsimony and BAMBE analyses.

From the Department of Haematology and Oncology, the assistance and advice of Dr Alexander Dobrovic, Dr Tina Bianco, Ms Tanya Sanders, Dr Sally Stephenson and Dr Jenny Hardingham were highly valued in the design and implementation of the methylation analyses in Chapter 5.

With deep gratitude, I acknowledge the support of several other valuable persons. Initially, I thank Mr Andrew Leppard for his friendship and encouragement during this PhD. Last, but not least, with much heart-felt gratitude I thank my mother Mrs Erika Varga and my father Mr István Varga for their continued love, patience, understanding and support during the course of this PhD.

List of Abstracts and Presentations

2003 **ComBio 2003 (Abstract)**
Melbourne, Victoria, Australia
Methylation Regulates Expression of *Uroplakin 1B* in Normal Tissues and in Bladder Cancer
Varga A.E., Leonardos L., Cowled P.A.

2002 **North Western Adelaide Health Services Research Day (Oral)**
Adelaide, South Australia, Australia
Identification of the Promoter for Human *Uroplakin 1B*
Varga A.E., Cowled P.A.

Australian Society for Medical Research Conference (Oral)
Adelaide, South Australia, Australia
Correlation Between Expression of *Uroplakin 1B* (*UPKIB*) and Methylation of the Promoter Region
Varga A.E., Sanders T., Dobrovic A., Cowled P.A.

14th Lorne Cancer Conference (Poster)
Lorne, Victoria, Australia
Methylation of CpG Sites in the Uroplakin 1B Promoter Correlates with Loss of mRNA Expression in Bladder Cancer Cell Lines
A.E. Varga, T. Sanders, A. Dobrovic, P.A. Cowled

2001 **North Western Adelaide Health Services Research Day (Oral)**
Adelaide, South Australia, Australia
Methylation of CpG Sites in the Uroplakin 1B Promoter Correlates With Loss of mRNA Expression in Bladder Cancer Cell Lines
Varga A.E., Sanders T., Dobrovic A., Cowled P.A.

Australian Society for Medical Research Conference (Oral)
Adelaide, South Australia, Australia
Genomic Organisation of *UPKIB*: a "Multi-Task" Approach
Varga A.E., Cowled P.A.

Genetics Society of Australia Conference (Poster)
Adelaide, South Australia, Australia
Evolutionary Analysis of Uroplakin 1B (UPKIB), a Member of the Tetraspanin Group of Proteins.
Varga A.E., Campbell H.D., Cowled P.A.

22nd Lorne Genome Conference (Poster)
Lorne, Victoria, Australia
Evolutionary Analysis of Uroplakin 1B (UPKIB), a Member of the Tetraspanin Group of Proteins.
Varga A.E., Campbell H.D., Cowled P.A.

2000 **North Western Adelaide Health Services Research Day (Oral)**
Adelaide, South Australia, Australia
Use of a P1 Artificial Chromosome in the Genetic Cloning of Uroplakin 1B, A Urothelial Protein.
Varga A.E., Cowled P.A.

Australasian Surgical Research Forum 2000 (Oral)
Surgical Research Society of Australasia
Adelaide, South Australia, Australia
Use of a P1 Artificial Chromosome in the Genetic Cloning of Bladder Uroplakin 1B, a Urothelial Protein.
Varga A.E., Cowled P.A.

Abbreviations

5'RACE	5' rapid amplification of cDNA ends
[α^{32}P]-dATP	α-deoxyadenosine phosphate labelled with ^{32}P
Amp	ampicillin
AUM	asymmetric unit membrane
bp	base pair(s)
˚C	degrees Celsius
cDNA	complementary DNA
DEPC	diethyl pyrocarbonate
DMEM	Dulbecco's modified Eagle's medium
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
dNTP	2'-deoxynucleoside-5' triphosphate
EST	expressed sequence tag
[γ^{32}P]-dATP	γ-deoxyadenosine phosphate labelled with ^{32}P
GenBank	nucleic acid and amino acid world-wide sequence resources compiled and maintained by NCBI
IPTG	isopropyl-β-D-galactosidase
Kan	Kanamycin
kb	kilobase(s)
kDa	kiloDalton(s)
LB	Luria Bertani media
LOH	loss of heterozygosity
μJ	micro Joule(s)
μl	micro litre(s)
mM	milli Molar(s)
MS-SSCA	methylation-sensitive single strand conformation analysis
NCBI	National Centre for Biotechnology Information, the National Library of Medicine, USA
nt	nucleotide(s)
ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
PBGD	porphobiliglobin deaminase
PBL	peripheral blood lymphocytes
PCR	polymerase chain reaction
RNA	ribonucleic acid
RNase	ribonuclease
rpm	revolutions per minute
RT-PCR	reverse-transcription polymerase chain reaction
SCC	squamous cell carcinoma
SDS	sodium dodecyl (laurel) sulphate
SSCP	single-strand conformation polymorphism
TCC	transitional cell carcinoma
U	units
UPK	uroplakin
V	volts
v/v	volume per volume
w/v	weight per volume

Some Variations of Correct Nomenclature

A. Uroplakin nomenclature

Several forms of nomenclature have been used for the uroplakin members, both in the literature and in the various Internet-based databases. These include (i) differences in 'uroplakin' abbreviation to 'UPK' or 'UP', (ii) differences in the uroplakins 1 through 3 numbering with Roman (I-III) or Arabic (1-3) numbering, and (iii) in the casing of a, A, b and B. For uniformity in this thesis, uroplakins 1 through 3 are numbered UPKIA, UPKIB, UPKII and UPKIII for all species.

B. Species abbreviations

Several names are used in simplified form for organisms. The organisms are:

Caenorhabditis elegans: Eukaryota; Metazoa; Nematoda; Chromadorea; Rhabditida; Rhabditoidea; Rhabditidae; Peloderinae; *Caenorhabditis*, *elegans*. Abbreviated to *C. elegans* or *Caenorhabditis*.

Drosophila melanogaster: Eukaryota; Metazoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Diptera; Brachycera; Muscomorpha; Ephydroidea; Drosophilidae; *Drosophila*, *melanogaster*. Abbreviated to *Drosophila.*

Manduca sexta: Eukaryota; Metazoa; Arthropoda; Hexapoda; Insecta; Pterygota; Neoptera; Endopterygota; Lepidoptera; Glossata; Ditrysia; Sphingiodea; Sphingidae; Sphinginae; *Manduca*, *sexta*. Abbreviated to *Manduca*.

Schistosoma haematobium, *Shistosoma japonicum*, and *Schistosoma mansoni*: Eukaryota; Metazoa; Platyhelminthes; Trematoda; Digenea; Strigeidida; Schistosomatoidea; Schistosomatidae; *Schistosoma*. Abbreviated to *Schistosoma*.

Xenopus laevis: Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Amphibia; Batrachia; Anura; Mesobatrachia; Pipoidea; Pipidae; Xenopodinae; *Xenopus*, *laevis*. Abbreviated to *Xenopus*.

C. Genetic terminology: homologous versus homoeologous

In Chapter 3 the word homologous is used; however, this is more exactly homoeologous if between different species. The term homoeologous is not often used in molecular biology.