Geoffrey A. Charters *MSc (Hons) PhD (Pathology)*

Molecular oncopathologist

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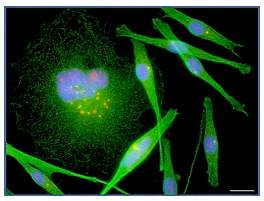

Curriculum vitæ

25 September, 2008

Qualifications

Doctor of Philosophy in Pathology

Institution: The University of Auckland, New Zealand 1998 – 2007; awarded 2008
Thesis: Human metastatic melanoma in vitro: ploidy, centrosomal integrity, serum dependency, chromosome 9p21 genetic status, tumour-suppressor gene expression.

Master of Science in Biological Sciences, Second Class Honours, Division 1

Institution: The University of Auckland, New Zealand 1995 – 1997; awarded 2008
Papers: Advanced Cellular and Molecular Biology for Biomedical Research, Molecular Genetics, and Cancer Biology
Thesis: Aspects of growth regulation in cultured human melanoma cells.

Certificate in Winemaking

Institution: Hawke's Bay Polytechnic, Napier, New Zealand 1993 – 1994; awarded 1994

Bachelor of Science

Institution: The University of Auckland, New Zealand 1978 – 1980; awarded 1984
Majors: Computer Science, Cell Biology, Biochemistry

Publications

Centrosomal dysregulation and nucleolar pericentrin reservoirs in human metastatic melanoma cell lines (manuscript in preparation)
Charters GA, Stones CJ, Finlay GJ, Shelling AN, and Baguley BC 2008

He Rauemi Rautaki Oranga Hinegaro / A Maori Mental Health Bibliographic Database
Manaia W, Bridgman G, Hatcher S, and Charters G
Health Research Council of New Zealand, Auckland New Zealand 2004

Effective models of crisis mental health service delivery
Hatcher S, O'Brien A, Coupe N, and Charters G
Health Research Council of New Zealand, Auckland New Zealand
ISBN 0-908700-13-X 2003

Radiation-induced cell cycle delays and p53 status of early passage melanoma cell lines
Parmar J, Marshall ES, Charters GA, Holdaway KM, Shelling AN, and Baguley BC
Oncology Research 12(3):149-5 2000

Symposium presentations

The response of human melanoma cells to serum deprivation
New Zealand Society for Oncology Annual Scientific Meeting Rotorua 2001

Aberrations of the *CDKN2A* gene in cultured human melanoma cells correlate with reduced, rather than increased in vitro growth rates
Human Genetics Society of Australasia Hamilton 1999

PCR-SSCP analysis of p16^{CDKN2A} gene status in human melanoma cell lines
New Zealand Society for Oncology Annual Scientific Meeting Wellington 1999

Attributes and skills

General

Intellectual

- Extremely strong inductive reasoning skills that enable robust hypothesis generation: the extraction of the relatively sparse salient elements from a large body of often internally inconsistent information and the formulation of a consistent model from them, while simultaneously identifying areas of conflict between the model and the unincorporated data, and any significant missing elements.
- Complementary deductive reasoning skills that allow the development of research or experimental methodologies to test or expand these theoretical models.
- An ability to perceive patterns in systems that can facilitate understanding through the use of analogies drawn from unassociated fields.

Communication

- Excellent written communication skills, with an ability to present complex material in a concise, precise, complete, consistent, clear, and orderly manner.
- An ability to conceive and produce complex diagrammatic representations of systems to aid in their description.
- Meticulous attention to details of spelling, grammar, and consistency of style.

Scientific

General

- Routine operation of materials handling/measuring systems: pipettes, balances, centrifuges, pH meters, spectrophotometers, fluorometers etc.
- Safe handling of toxic, mutagenic, corrosive and biohazardous material.
- Good basic understanding of statistical methods: linear regression, hypothesis testing.

Cellular and molecular biology

- Familiarity with online tools and resources: Pubmed, OMIM, BLAST, Primer3, etc.
- Preparation of routine cell culture solutions and reagents for experimental procedures.
- Routine maintenance of human cells in serial passage culture.
- Storage of and retrieval from cryogenic storage of viable human cells.
- Phase contrast, fluorescence, and laser-scanning confocal microscopy.
- Flow cytometric determination of ploidy and cell cycle phasing.
- Flow cytometric determination of protein expression.
- Isolation, purification, and concentration of high quality DNA, RNA, and protein.
- Polymerase chain reaction design, optimisation, and execution.
- Agarose and acrylamide gel electrophoresis of nucleic acids.
- Acrylamide electrophoresis and Western blotting of proteins.
- Preparation of PCR products for sequencing.
- Analysis of DNA sequencing and microsatellite analysis results.
- Restriction and ligation enzyme procedures.
- Bacterial transfection of PCR products.

Computational

Applications

- Highly proficient user of Apple Macintosh systems and standard software.
- Extensive knowledge of Microsoft Office applications.
- Good working knowledge of Adobe Photoshop.
- Familiarity with a great many other applications and utilities.

Programming

- Formerly, an expert with PASCAL and Macro-11 assembler for RSX11.
- Fair competency with Visual Basic for Applications.
- Some skill with Java, Objective-C, Javascript, DHTML, XHTML, PHP, JSP, MySQL.

Miscellaneous

- Dual citizenship: New Zealand by birth; United Kingdom by descent.
- Full N. Z. car driver licence.
- Significant knowledge of: science and technology in general; 35 mm film SLR and DSLR photography; native N. Z. flora.
- Excellent cook.

Employment history

Film and Television Extra (part-time) 2003 – present

Agencies: August Models and Talent, Hidden Taonga/Café Photo, Visage Models and Talent

Credits:
- TV series: *Mercy Peak*, *Shortland Street*, *Outrageous Fortune*.
- Miniseries/movies *Hercules*, role of "Argus"; *Skin and Bone*.
- Music videos: *Brothaz* (Nesian Mystic), *Without You* (Minuit).
- TV advertisements: numerous and various, at least ten in total

Various casual, part-time, or short-term positions Dec 2002 – 2005

Employer: The University of Auckland

Positions:
- Research assistant (Department of Psychiatry).
- Examination marking (Department of Anatomy).
- Laboratory demonstration (Department of Molecular Medicine and Pathology).
- Database programmer (Department of Psychiatry).
- Examination assistant supervisor (Faculty of Medical and Health Sciences).

Science Coördinator and Teacher (part-time) Jun 2003 – Dec 2004

Employer: The George Parkyn National Centre for Gifted Education

Duties:
- Preparation and delivery of intensive one-day single-topic lessons to gifted children in the 8 – 12 year age range within the context of the One Day School operated by the centre. Topics included: *Topology and Space-time*, *Boolean Algebra and Digital Electronics*, *The Electromagnetic Force*, and *The Central Dogma of Biology*.

Software Synthesist, Process Information Controller (full time) Oct 1983 – Aug 1994

Employer: Nelsons (NZ) Ltd; Weddel-Crown Corporation (NZ) Ltd; Weddel (NZ) Ltd

Duties:
- Completion and further development of the data acquisition and marshalling system referred to below including significant extensions to functionality, alterations due to changing requirements, operator training, system documentation, system migration to new hardware/operating systems, close liaison with engineering maintenance staff, emergency fault finding and remedy.
- Training of over 100 staff members in the rudiments of computing, with a core of approximately 10 proceeding to elementary programming.
- Plant-wide network advocacy, planning and implementation.
- Completion of a highly detailed plant-wide product-flow analysis as a precursor to the implementation of an inventory control system.
- Development of a computer-based cold-store operations simulator used at two of the Groups sites.
- Supervision and direction of the Small Systems Support Team responsible for PC application and network support.
- Participation in the Plant Technical Steering Committee.
- Representation of the Group at the Meat Industry Association Technical Committee.

Computer Programmer / Systems Analyst (full time) Jan 1981 – Oct 1983

Employer: Automation Engineering (a division of Refrigeration Engineering Ltd)

Duties:
- Design and implementation of real-time data-acquisition and analysis software for industrial applications.

Context:
- Hardware platforms included Digital Equipment Corporation PDP11 family processors under the RSX11M/S operating system and Motorola 6800 family microprocessors under the AEROS kernel.
- Programming was in PASCAL and MACRO-11 assembler for PDP11 and AERIAL structured assembler for M6800.

Projects:
- Programming a microprocessor-based irrigation controller for horticultural applications.
- Modifications to the control software for the Mangere sewage treatment plant.
- Most significantly, software design and implementation for a 30 processor hierarchically-networked data acquisition and marshalling system for the Mutton Complex at the Tomoana Freezing Works in Hastings.

Awards

Biomedical Imaging Reseach Unit Awards (University of Auckland) 2006

- Award for Best Light Microscopy Image
- Trophy for Best Image Overall (see letterhead)

Referees

Professional

Professor Bruce C. Baguley ONZM MSc (Hons) PhD FRSNZ
The Auckland Cancer Society Research Centre (Co-director)
The University of Auckland, New Zealand
Phone: + 64 9 373 7999
Email: b.baguley@auckland.ac.nz

Associate Professor Andrew N. Shelling BPhEd BSc (Hons) PhD
Medical Genetics Research Group (Head)
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Character

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Thesis Abstracts

Doctor of Philosophy

Metastatic melanoma cells frequently have flaws in the proliferative control mediated by the retinoblastoma-associated protein (pRB), normally operative at the G_1-S cell-cycle phase transition. Functional and molecular aspects of this were investigated in seventeen human metastatic melanoma cell-lines (NZMs).

Flow-cytometry revealed aneuploidy and heteroploidy in many NZMs that were unstable over time. This plasticity may contribute to melanoma's therapeutic resistance. A potential cause of this, dysregulation of centrosomal numerical control, was demonstrated by immunofluorescence microscopy, apparently for the first time in melanoma cell-lines.

Pericentrin was found to accumulate in nucleolar reservoirs, previously unreported, and release from these on nuclear envelope breakdown may trigger mitotic spindle formation. Dysregulation of pericentrin may be particularly important in melanoma, and could represent a therapeutic target.

To test the integrity of pRB-mediated proliferative regulation, NZM cells were grown under conditions of serum deprivation that in normal cells would cause arrest via pRB. Cell-cycle phase analysis revealed three classes of response: accumulation in G_1, accumulation in G_2 or mitosis, or stimulation to enter S phase. G_1 arrest indicates that proliferative regulation in response to serum deprivation may be normal in some melanomas, implying that the pRB subsystem may not be the sole regulator of this, or that it is not defective in all melanomas.

Using PCR deletion analysis, an investigation was undertaken into the integrity of the tandem 9p21 *CDKN2A* and *CDKN2B* genes that encode tumour-suppressors implicated in melanoma tumorigenesis. Homozygous deletions affecting only *CDKN2A* were found in two cell-lines, and affecting both genes in six. In the case of NZM7, where different sub-clones exist, heterogeneity was found by microsatellite analysis. DNA sequencing revealed a known *CDKN2A* `G500C` polymorphism in the NZM7 group, also heterogeneous among sub-clones. A *CDKN2B* `G411A` polymorphism was found in NZM14, but it is predicted not to affect the amino acid sequence of the encoded protein.

Protein analysis revealed that all NZMs express pRB, but in some, this was in the inhibitory unphosphorylated state, despite their being proliferative. This correlated with strong p16 expression and known *BRAF* mutations, suggesting that proliferation of *BRAF* mutants may require compromised function of the pRB subsystem.

Master of Science

The work at hand describes investigations undertaken between October 1995 and February 1997 at the Cancer Research Laboratory of the New Zealand Cancer Society (Auckland Division). The intention was to explore the different sensitivities of the human melanoma cell-lines NZM4 and NZM7 to growth inhibition by the cytokine TGF-β.

Several TGF-β resistance mechanisms were considered. One in particular became prominent. In TGF-β-treated mink lung epithelial cells cyclin-dependent kinase four (cdk4) is translationally down-regulated in a p53-dependent manner. In unrelated work, evidence was found for a pathway leading to G_1 arrest after irradiation that depended upon p53, but not $p21^{CDKN1}$, the established route. Together, these reports suggested that the same mechanism may be involved in both cases.

Flow cytometric analysis of the G_1 cdk4 content of cultures revealed no evidence for down-regulation in response to TGF-β, etoposide or bleomycin. Surprisingly, indications of dose-dependent down-regulation were found in NZM7 after irradiation but this was not associated with the proportion of G_0-G_1 cells and cannot be causal.

Strong evidence was found of a minimum cdk4 concentration threshold that must be surpassed before entrance to S phase implying that cdk4 is rate-limiting for cycle progression in these cells. As no increase in cdk4 concentration was seen during S phase, the implication is that immediately after mitosis insufficient cdk4 will be present to sponsor immediate re-entry to S phase and consequently the occurrence of a G_1 phase is assured.

This minimum level was found to persist throughout S phase, an observation that opposes the hypothesis that cyclin D-cdk4 is required only for S phase entry. A correlation was found between reduction in cdk4 levels and the onset of chromatin condensation before mitosis and there were indications that this may be a prerequisite for such condensation but the data were inconclusive.

In all, a great deal was learnt about laboratory procedure particularly concerning the maintenance of human cell-lines in tissue culture and techniques of flow cytometry.

Several novel observations are reported: down-regulation of cdk4 in response to irradiation, maintenance of a minimum cdk4 level throughout S phase, and the association between cdk4 down-regulation and chromatin condensation.