2001 Dr. Jorge Sergio Reis Filho, Portugal

REPORT OF ACTIVITIES OF GORDON SIGNY FOREIGN FELLOWSHIP IN PATHOLOGY

JORGE SERGIO REIS-FILHO

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Jorge Sergio Reis Filho, MD, PhD

Report of activities of Gordon Signy Foreign Fellowship 2001 – 2005, WASPaLM (World Association of Societies of Pathology and Laboratory Medicine) and WPF (The World Pathology Foundations)

Institutions

The Breakthrough Breast Cancer Research Centre, Institute of Cancer Research, London, UK

Institute of Molecular Pathology and Immunology, University of Porto, Protugal

Supervisors:

Prof Sunil Lakhani

Prof Alan Ashworth

Prof Fernando Schmitt

July 2002 to August 2005

Supervisors: Prof Alan Ashworth, Prof Sunil R Lakhani and Prof Fernando C Schmitt.

In August 2002, I started my clinical research fellowship in molecular breast cancer pathology under the supervision of Prof Sunil Lakhani, Prof Alan Ashworth and Fernando Schmitt. The project focused on myoepithelial/ basal differentiation in breast carcinomas, with a special emphasis on basal-like and metaplastic breast carcinomas. I was actively involved in the analysis of the expression of immunohistochemical markers performed on tissue microarrays and also learnt various molecular pathology techniques, including laser capture microdissection, cDNA microarrays and comparative genomic hybridisation. Together with Dr Peter Simpson, Dr Chris Jones and Alan Mackay, I developed a method for microarray-based comparative genomic hybridisation analysis, which can be performed with

DNA extracted from frozen and paraffin-embedded tissues. This resulted in the publication of manuscripts in pathology and cancer-related journals (1-7). In fact, the training in high throughput molecular methods was comprehensive and I can now plan, set up and analyse cDNA/ oligonucleotide expression profile experiments, comparative genomic hybridisation assays and microarray-based comparative genomic hybridisation analysis.

In collaboration with the Breast Cancer Linkage Consortium, I had the chance of studying the expression of basal and myoepithelial markers in familial breast carcinomas and demonstrated that the expression of 'basal' cytokeratins can help identify patients with BRCA1 germline mutations. These findings were published in Clinical Cancer Research in 2005 (8).

From August 2002 to September 2004, together with the other clinical fellow, Dr Laura Fulford, I handled the referral cases sent to Prof Sunil Lakahni. This involved the histopathological and immunohistochemical analysis of the cases. This resulted in the publication of two "*Residents' Pages*" (9, 10) in the Archives of Pathology and Laboratory Medicine. I also did the macroscopical and histopathological analysis of mammoplasty specimens sent to The Breakthrough Breast Cancer Research Centre, which were then signed out by Prof Lakhani.

Following Prof Lakhani's departure in September 2004, my diagnostic activities were restricted to those involving the mammoplasty specimens under the supervision of Dr Ashutosh Nerukar; however from that moment on, I became responsible for all the pathological analysis of all animal models generated at the Breakthrough Centre, provided an antibody optimisation service for teams within the Institute and helped in the interpretation of pathology specimens for translational research projects.

I established, in collaboration with the histopathology core facility, a new tissue array from an existing database of patients treated with anthracycline-based chemotherapy at the Royal Marsden Hospital, including selecting and assessing a baseline set of immunohistochemical markers. These tissue microarrays have been used at the Breakthrough Breast Cancer Research Centre as a tool to investigate the prognostic impact of several genes and proteins identified in expression profile, microarray-based comparative genomic hybridisation and siRNA screening studies.

In addition, I developed a method for generating probes for chromogenic and fluorescent *in situ* hybridisation, which has proven crucial not only for the validation of results obtained with microarray-based comparative genomic hybridisation, but also for the study of copy number changes of genes of interest (11).

August 2005 – March 2006

Supervisor: Prof Fernando C Schmitt

In August 2005, I returned to Portugal, where I continued working under supervision of Prof Fernando Schmitt as an 'Associated Investigator' at the Institute of Molecular Pathology and Immunology, University of Porto, Portugal.

Given the nature and novelty of the work I carried out whilst at the Breakthrough Centre, Prof Fernando Schmitt and the post-graduation studies committee of the University of Minho, Portugal, considered the body of work generated from July 2002 to August 2005 sufficient to award me a higher degree.

A doctoral thesis was written up and I had my viva on 7th March 2006, when I was awarded a PhD degree, which received the maximum score.

Soon after, I received a job offer at the Institute of Cancer Research to lead the Molecular Pathology Laboratory. Currently, I have a permanent position at Faculty Level with the Institute of Cancer Research, London, UK and a honorary contract with the Institute of Molecular Pathology and Immunology, University of Porto, Portugal.

Summary of Techniques Learnt

Following my training at the Breakthrough Breast Cancer Research Centre, Institute of Cancer Research, London, I have become fully conversant in the techniques below:

- Expression profile analysis using in-house cDNA spotted arrays: development of microarray chips, hybridisation protocols and data analysis using GeneSpring
- Expression profile analysis using oligonucleotide arrays (Affymetrix): data analysis
- Comparative genomic hybridisation – Vysis system and Applied Imaging International system: hybridisation protocol and metaphase spread analysis
- Microarray-based comparative genomic hybridisation with spotted bacterial artificial chromosomes: platform development, protocol implementation for both fresh/ frozen and paraffin embedded samples
- Fluorescent *in situ* hybridisation: probe generation and hybridisation protocols for cell lines, frozen samples and formalin-fixed, paraffin embedded specimens

- Chromogenic *in situ* hybridisation: probe generation and hybridisation protocols for cell lines, frozen samples and formalin-fixed, paraffin embedded specimens
- Loss of heterozygosity analysis: protocols for analysis of formalin-fixed, paraffin-embedded samples
- Methylation-specific polymerase chain reaction assays: protocols for analysis of fresh/ frozen and formalin-fixed, paraffin-embedded samples
- Tissue microarrays: implementation and construction of tissue microarrays; immunohistochemical analysis of tissue microarrays; fluorescent and chromogenic in situ hybridisation analysis of tissue microarrays; bioinformatic analysis of tissue microarray data, including hierarchical clustering analysis

Most importantly, the continued supervision offered by Prof Lakhani and Prof Ashworth have enabled me to learn how to interpret the results of molecular techniques and how to put them in context with pathological findings (12, 13). This has significantly improved my scientific thinking in general and changed my perception of molecular data available in the literature.

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