History of Cytogenetics at St Vincent's Hospital Melbourne

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The history of the Cytogenetics department at St. Vincent's Hospital (SVH) in Melbourne begins with Dr O. Margaret Garson, who, in association with Professor Albert Baikie, started it as a research interest in the University of Melbourne Department of Medicine (DOM) at SVH in the 1960's, saw it recognized as a separate department in the 1980's and still maintains ties with the department today.

Margaret graduated from Medicine at the University of Melbourne in 1951 and became a resident at the Alfred Hospital where she had been a student. At that time it was not easy to advance in Medicine if one was a woman. Because of her gender, she was denied further training in Obstetrics which was what she thought she wanted to do. There was no such thing as a law against sexual discrimination. So, she changed her plans and managed to get accepted into training in Pathology. She trained in Pathology and Haematology and met her husband over the autopsy table in the Pathology department at the Alfred Hospital.

Margaret was led into cytogenetics by a lucky accident. In 1959, her husband was awarded a scholarship for a three year stay at the University of Texas Medical Branch in Galveston to study burns management and plastic surgery. At this stage, Margaret was a trained haematologist but her visa was as an accompanying person which prohibited her from working on pain of deportation. Her husband's boss's wife was a well-connected doctor who managed in a short space of time to organise a job for Margaret as a research fellow in the

Research Haematology Department, obtain an NIH grant for her and arrange for a maid to take care of her children. The visa was the easiest of all. She paid $25, filled out a form and it was done.

The Director of the Research Haematology Department was Dr. W.C. Levin who had recently read of the discovery of the Philadelphia chromosome by Nowell and Hungerford and thought it might be pertinent to learn about chromosomes and perhaps to get someone to introduce chromosome studies into his laboratory. Because Margaret was considered a morphologist, he decided that she should be that person and sent her to the Rockefeller Institute in New York to the laboratory of Dr Jim German, to learn chromosome techniques. At this stage she knew very little about the subject, apart from the fact that there were 48 chromosomes in the human chromosome complement, a fact that she had been taught as a medical student and which she soon discovered was incorrect. So Dr Levin suggested that before she went to New York she should deliver a paper to the Haematology Research staff on the topic of cytogenetics in haematology. This was 1961 and Margaret read what little available literature there was on the subject. It was then she discovered that, in the late 1950's, it had been shown that the human possessed 46 chromosomes in all cells, and as she read on, the subject became more and more fascinating. In a Carl de Gruchy Memorial Lecture, she once compared it to Alice in Wonderland's fall down the rabbit burrow and her arrival in the hall where she was surrounded by a large number of locked doors and how the unlocking of these doors revealed exciting possibilities beyond.

The first of these doors to be opened was by a person who became an old friend, Dr T.C. Hsu, who headed the Department of cell biology at M.D. Anderson Hospital in Houston,

Texas at the time. He was the one who in 1956 enabled the exact count of 46 chromosomes in the human to be made because of a monumental and yet chance discovery. TC was an insect geneticist who came to America from China in 1948. He was actually working on the campus of the University of Texas Medical branch in Galveston when he happened on the finding that revolutionised the visualisation of human chromosomes. He wrote a book in which he described this: he was working on a few samples of foetal tissues that had been sent to the laboratory. In those early days many cultures from human tissue were set up in an attempt to obtain even a single adequate mitosis capable of analysis, as the chromosome morphology was so poor. He set up skin and spleen cultures in the usual way, fixed some of the cultures and stained them with haematoxylin. He could just not believe his eyes when he saw, down the microscope, some beautifully scattered chromosomes within these cells.

Characteristically, he didn't tell anyone, he took a walk around the building went to the coffee shop and then went back to the lab and had another look and the beautiful chromosomes were still there, they hadn't gone away and he knew they were real. So he set about trying to decide why. He prepared some more cultures the next day and waited for the miracle to happen again, but it didn't. The mitotic figures were there but they had resumed their normal miserable twisted unidentifiable appearance so he repeated it again and again and still nothing happened. He began to think that perhaps there was something unusual about the particular set of human splenic cultures which made the mitotic cells so marvellous. So for about three months he altered every factor that he could think of in the culture: the medium components, the culture conditions, the incubation temperature and different amounts of colchicine, a substance which had been found by plant cytogeneticists to arrest cells in metaphase. He altered his fixation procedures, his staining methods and nothing worked, until he changed the tonicity of the balanced salt solution which was commonly used

in the laboratory to rinse the cultures before fixation. When he did this and looked down the microscope the miracle had reappeared. He said it was certainly a wonderful feeling to be able to solve a mystery and he realised that he had a powerful tool in his hands. He set about testing this tool against materials other than human cells and he found that using this hypotonic solution to rinse out the colchicine produced beautiful chromosomes in all species and in all cultures. Only then did he realise that the first set of human splenic cultures which had given him the magnificent mitotic figures had been accidentally washed in a hypotonic solution. He said the only logical explanation was that one of the technicians who had prepared the balanced salt solution had misread the scale and prepared it as a hypotonic solution without knowing it and since nearly four months had elapsed from the time of the well spread chromosomes there was no way to trace who had actually prepared that particular bottle, but he commented in his book "Even today I would love to give a peck on the cheek to that lady who made an important contribution to cytogenetics without knowing it".[1]

TC became Margaret's mentor when she returned to Galveston from the Rockefeller Institute and attempted to produce chromosomes of her own. Although chromosome preparations are now made in cytogenetics laboratories all over the world without a second thought, it took her over three months before she could produce anything in the way of decent chromosomes and in those frustrating three months she beat a track the sixty miles up the freeway from Galveston to Houston to TC's laboratory.

The next major event in the study of human cytogenetics was the discovery of the effect on lymphocytes of phytohaemagglutinin. In 1960 Peter Nowell, a pathologist working at the University of Pennsylvania, was studying leukocytes in culture. After several days of

incubation he noticed that he had a large number of blast-like cells and mitoses in his cultures. He couldn't explain this and thought that there must be some factor in the tissue culture medium causing this unusual phenomenon, because in those days leukocytes were considered to be end products of cell developmental lines and unable to undergo mitosis. He, like TC, looked at all the different components in the medium to see what might be causing this effect, with no result. He was separating the leukocytes from the red cells prior to cell culture by adding phytohaemagglutinin which is as its name implies an agglutinator of red cells. This was a substance derived from the common navy bean or phaseolis vulgaris. Nowell found that if he did not use phytohaemagglutinin to separate the white cells prior to culture he did not get any mitoses, so it became obvious that phytohaemagglutinin contained a stimulating factor which stimulated lymphocytes to divide in culture. He submitted a manuscript to Cancer Research describing this discovery and recalled the comment from the Journal: "it is an interesting observation but of no conceivable significance to science". Of course, his discovery now ranks as one of the milestones in human cytogenetics.

Prior to this discovery, all chromosome analysis had been carried out on cells actively dividing in the body, such as fibroblasts. It now became possible to perform chromosome studies merely by removing a small quantity of blood from patients and incubating it in a nutritious medium together with the phytohaemagglutinin. In two to three days, a predictable number of these cells had gone into mitosis, could be arrested by colcemid, swollen by the hypotonic solution and chromosome preparations made. This made it so easy to investigate the chromosome constitution of individuals that many laboratories throughout the world started performing chromosome analyses. The demand for phytohaemagglutinin became so high that available stocks were soon depleted and many laboratories set about brewing their

own phytohaemagglutinin. In TC's laboratory, there were always large stone jars in which solutions of beans were brewing away producing phytohaemagglutinin.

One of Margaret's mentors during this period in Galveston was Jim German. He was one of the pioneers in human cytogenetics who had shown, in the early 1960's that at least some of the human chromosomes could be recognized individually by their different replication times. He accomplished this by a very time consuming procedure of incorporating radioactive thymidine into blood cultures and preparing auto-radiographs of the mitotic spreads of the chromosomes. He established a laboratory first at the Rockefeller Institute and later at the Blood Transfusion Centre in New York where he became interested in the chromosome damage found in the circulating cells of patients with Bloom's syndrome and later Fanconi's anaemia, both conditions known to have an increased incidence of leukaemia. These observations opened the eyes of many to the group of diseases we now call diseases of DNA repair and we have learnt a considerable amount about chromosome behaviour by a study of these rare autosomal, recessively inherited disorders. Jim's particular interest became Bloom's syndrome and the characteristic appearance of the chromosomes. He kept the world registry of Bloom's syndrome and would never support the diagnosis if recombinant chromosome figures were not present. He used to travel the world confirming the diagnosis and he quite often asked why Margaret couldn't find one in Australia because he always wanted to visit this country. When she finally did find one, unfortunately his travel money had run out at a total of fifty patients and hers was number fifty-three.

Another important mentor was Albert Baikie. He was a gentle and kindly person but a great force in cytogenetics both in Australia and internationally. In Mexico City, in 1962, when

Margaret was attending the International Society of Haematology meeting, she renewed acquaintance with Professor Carl de Gruchy, Professor of Medicine at SVH, who was busily inviting people to come to the next International Meeting to be held in Sydney in 1966. He was interested to learn that Margaret had been attracted to cytogenetics and suggested that when she returned to Australia she should come and see him at the DOM at SVH in Melbourne because he hoped that by that time Albert Baikie from Edinburgh would be working in the Department. Margaret was already familiar with Albert Baikie's name from her readings. Albert had been associated with quite a few early publications in haematological cytogenetics. He had gone into the science in the same way as Margaret, via haematology, and but for the difference in publication times in overseas journals the Philadelphia chromosome could just as readily have been called the Edinburgh chromosome. Nowell and Hungerford found the small abnormal G group chromosome in the bone marrow cells of patients with chronic myeloid leukaemia at more or less the same time as Baikie, Court-Brown and Jacobs in Edinburgh but the group working in Philadelphia wrote a letter to Nature which was published just prior to the communication that was to be sent off from Edinburgh. So, the chromosome has gone down in posterity as the Philadelphia chromosome. This finding in 1960 of a specific chromosome abnormality which was constantly associated with a malignant disease was the first breakthrough in human cancer and haematological cytogenetics and set the stage for what was to come in the next 20 years.

Margaret and her family returned to Australia in late 1963 and in 1964 she took up the post of research associate to Albert Baikie, who had joined the DOM as the new first assistant in 1963. The two of them became the core of a cytogenetics laboratory in the DOM. They were joined soon after by a scientist, Sandra Weste (now Heard), who came to work on a research project for which Albert had been successful in obtaining funding from the then Anti-Cancer

Council of Victoria (ACCV). Sandra subsequently went on to head the cytogenetics laboratory at the Royal Hobart Hospital. The research project was to study the incidence of X chromosome abnormalities in a general hospital population as discovered by buccal smear and confirmed by cytogenetic analysis, and also to see if there were any disease association. Sandra, Gill (a technician) and Margaret set about the task of taking buccal smears from every patient admitted to SVH over a 3 year period and collected results from 21,364 patients. They obtained the services of Pat Walsh, an SVH nurse and buccal smear rounds were done twice a day on weekdays and daily on weekends and holidays. It was an interesting study, identifying 19 patients with Klinefelter Syndrome (47,XXY) and 9 with Triple X and showing an association of varicose veins with 47,XXY.

With chromosome analysis made easy and within the reach of any interested person, the early 1960's produced a flood of discoveries in human cytogenetics. Chromosome abnormalities were found in acute leukaemia cells, in some patients with polycythaemia and myelfibrosis and in solid malignancies but apart from the Philadelphia chromosome in CML the abnormalities identified in these various disorders did not appear to fall into any particular pattern and were thought at that stage to be pure epiphenomena in the development of malignancy, of little use in diagnosis or prognosis.

All changed with the discovery by Caspersson, a Swedish expert in fluorometry and interferometry at the Karolinska Institute, who identified a method of visualising banding patterns that were specific for each chromosome. Caspersson argued that if an alkylating agent could be attached to a fluorochrome molecule, the alkylating agent might cross link the guanines of the DNA and the stain would give a fluorescent signal at the site of attachment. If

the distribution of the base pairs along a chromosome was non-random, the chromosome would show differential fluorescence along its length and individual chromosomes might show different fluorescence or banding patterns. He applied his theory first to plants before turning his attention to human chromosomes and found that he could produce banding patterns across the chromosomes. This became known as Q-banding because the fluorochrome used was a quinacrine derivative. Following this discovery of Caspersson's many workers started looking for other ways of identifying the chromosomes with banding techniques. Q-banding needed ultra-violet light for its identification and the fluorescence was very pale and faded quickly. Therefore, more permanent banding preparations were needed and the answer to this was G-banding, where chromosomes are treated by different techniques: either a solution of an enzyme such as trypsin or incubation in a hot salt solution and staining the resultant preparations with Giemsa, to produce Giemsa or G-bands.

Following the initial development of G-banding, there appeared an explosion of publications on better and brighter ways of producing G-bands using anything from enzymes to potassium permanganate, even detergent, and many other types of banding were described e.g. C-banding which stains the constitutive heterochromatin of the chromosomes, R-banding which produces the reverse of G-banding, T- or terminal banding which stains the ends of the chromosomes and so on. All this was aptly termed by T.C. Hsu in his book, the "band wagon".

This was the new era in cytogenetics. The first exciting discovery was made by Janet Rowley in Chicago, who discovered that the Philadelphia chromosome was not caused by loss of part of the long arm of one chromosome 22 but that part of 22 was now attached to the bottom of

one chromosome 9; the small Philadelphia chromosome was due to a translocation between the chromosomes which could obviously lead to the repositioning of genes and the creation of at that time unknown genetic effects.

At SVH, in 1966, Albert Baikie left to become the first Professor of Medicine at the University of Tasmania, Sandra left to start a family and Margaret inherited the cytogenetics laboratory. From the 1960's, the ACCV (now the Cancer Council of Victoria) has supported the research work of the laboratory, which consisted initially of Margaret and one technician. Gradually, more staff and more research projects were acquired with funding obtained from the ACCV, Cancer Institute, University of Melbourne, SVH and the National Health and Medical Research Council among others. Throughout these early years, Professor Carl de Gruchy provided tremendous support and encouragement both for Margaret and for the development of the department.

One of the research staff employed was Wendy Milligan who was experimenting with various G banding preparations whilst Margaret was overseas in 1970 visiting Janet Rowley. When Margaret returned, Wendy excitedly showed her the bone marrow of a patient with a Philadelphia chromosome and a missing Y chromosome on whom she had tried G-banding. She identified a little tag on the bottom of chromosome 9, suggesting that this might be the Y chromosome. Margaret presented these findings at a departmental seminar and a person in the audience who had recently returned from a research meeting in Atlantic City where Janet Rowley had presented her exciting findings, told her that she had just missed discovering the Philadelphia translocation.

During the 1970's, journals became flooded with descriptions of banded chromosome abnormalities in leukaemia and these abnormalities were found to have morphological and prognostic associations. The International Workshops on Chromosomes in Leukaemia were born in 1976 and Margaret was invited to be the Australian representative. This involved much work collecting data but, as by this time, the cytogenetics laboratory at SVH had become the centre for cancer cytogenetics in Australia, Margaret was able to provide many interesting cases for discussion at the Workshops held in Helsinki, Leuven, Lund, Chicago, Tokyo and London.

In 1976, the Hospitals and Charities Commission recognized the diagnostic aspect of the work and formalised Margaret's appointment as Director of the unit within St. Vincent's Hospital. Over the years, many members of staff came and went but some came and stayed. In 1978, Trish Michael started as a research scientist studying the cytogenetics of acute lymphoblastic leukaemia. She was appointed to the hospital scientific staff in 1985 and remains on staff, as a Grade 3 Scientist and head of training, to this day. Fran O'Malley started work in the department in 1980 as a temporary replacement staff member and is, today, the Principal Scientist of the department.

As the department at SVH grew, Margaret helped to advance the science of cytogenetics in Australia. In 1966, Albert, Sandra and Margaret ran a workshop on human chromosomes, mainly focussing on stimulated peripheral blood chromosomes, but also touching on bone marrow cytogenetics. From 1985, Margaret, with the help of the departmental staff, ran a

series of bone marrow chromosome workshops to train Australian scientists in laboratory techniques and most of the scientists in laboratories around Australia at that time attended these workshops, establishing useful links for future co-operation.

The Cytogenetics Department was physically located within the DOM on the third floor of the Clinical Sciences Building in a series of small rabbit warren like rooms until 1988 when it moved into a purpose built laboratory space on the third floor of the old Pathology Building, the space previously occupied by the St Vincent's Institute of Medical Research. That same year, Dr Lynda Campbell, a trained haematologist as Margaret had been, started as a Fellow in the department, funded by a Keogh Fellowship from the ACCV. Lynda's interest in cancer cytogenetics had been stimulated by attending a session of the 1986 International Society of Haematology meeting in Sydney given by Dr Janet Rowley and Dr Carlo Croce on cancer cytogenetics and the cloning of genes involved in some of the common translocations observed in leukaemia and lymphomas. Lynda had completed her Haematology fellowship in 1986 and commenced training in cytogenetics in 1988 at SVH, with a year in Philadelphia in Dr Carlo Croce's laboratory in 1990. She returned to Melbourne in 1991, sat her Fellowship of the Human Genetics Society of Australasia in 1992 and, following Margaret's retirement, was appointed as Director of the Cytogenetics Department in October 1992.

At the time of her 65th birthday in 1992, Margaret's retirement from SVH was marked by a festschrift that was attended by cytogeneticists and haematologists from all over Australia and around the world, including cytogenetics colleagues from the UK, Sylvia Lawler and Lorna Secker-Walker who flew from London, and Michael Keating, a distinguished alumnus of the SVH DOM in the 1960's, who arrived from the M.D. Anderson Cancer Centre in

Houston, Texas. The outstanding array of speakers and attendees was a measure of Margaret's standing in the national and international cytogenetics community.

The early 1990's was a time of considerable change at SVH. The last Sister Administrator had been replaced by a Chief Executive Officer and fiscal responsibility was the order of the day. Since 1988, when the small cytogenetics laboratory at the Royal Melbourne Hospital closed its doors, the Cytogenetics laboratory at SVH had operated as a state referral centre, receiving specimens from every hospital in Victoria and both public and private laboratories. Only a fraction of its workload was derived from SVH patients. The hospital administration was therefore keen to establish a more equitable system of payment for the services of the department. In 1993, Dr Michael Stanford, the Director of Medical Services, petitioned the State Department of Human Services successfully for block funding to support the work of the department. With this change in funding source, the department was renamed the Victorian Cancer Cytogenetics Service (VCCS), to reflect formally its recognition as a state reference centre.

At this time, SVH was engaged in a major building project. The old Pathology building was levelled to the ground and the VCCS moved, along with most of the other Pathology services on the SVH campus, to the second floor of the new Inpatient Services Building towards the end of 1995. Over the next 15 years, the department increased its workload dramatically, from receiving approximately 1500 specimens in 1994 to more than 5,500 specimens in 2010. The staff of the department also expanded to keep pace with the ever increasing workload and, in 2007, Dr Meaghan Wall, a haematologist who had completed her PhD at

Peter MacCallum Cancer Centre, joined the department as a Clinical Fellow, supported by a Victorian Cancer Agency Clinical Research Fellowship.

In keeping with its earlier role in the training of cytogeneticists around Australia, over the last few years, the VCCS has hosted a number of visiting scientists from Malaysia, Singapore and India. These scientists have been trained in cancer cytogenetics and been able to take these methods back home, expanding the availability of cancer cytogenetics throughout Asia. Lynda has been a member of the International Standing Committee on the Human Cytogenetic Nomenclature since 2001 and a member of the International Working Group on Cytogenetics of Myelodysplastic Syndromes since 2007, maintaining the links of the VCCS with the international cytogenetics community, just as Margaret had done since the inception of the department as a research interest of the SVH Department of Medicine in 1964.

1. Hsu TC. *Human and Mammalian Cytogenetics: An Historical Perspective*. New York: Springer-Verlag; 1979.