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CANCER RESEARCH IN MANHATTAN

R. Leung, 24/15 East Parkside, Edinburgh EH16 5XN

My elective provided me with a chance to explore a field of medicine which I had found particularly interesting. Cancer is a dreadful disease and affects people from all walks of life and of all ages. Fascinated by the biology of the disease and touched by the many sufferers I had come across, I chose to do a laboratory project in a specialist centre in New York. This was unconventional and a far cry from the clinical elective which most medical students favour and turned out to be an eye-opening experience.

My accommodation was on the campus of the Rockefeller University, renowned for postgraduate education and medical research, a stone's throw away from my place of work. Filled with *Asyllia* blossom in spring, it was an oasis amidst the hectic street life of Manhattan and it also became the perfect base for me to discover what New York really has to offer. The buzzing metropolis with its streets filled with frantic activities was my birthplace, but I was taken away aged one month. So this was a wonderful chance to get acquainted with this beautiful and interesting city, the myriad of art galleries, the concert halls at Lincoln Centre and the theatres, and I even managed to trespass a few film sets. Not only is it a city full of life and vitality with endless offerings waiting to be tried, it was to my surprise very hospitable.

The Memorial Sloan-Kettering Cancer Centre

I was to work in the Memorial Sloan Kettering Cancer Centre (MSKCC). The Centre's emblem signifies their goals: to make continued advancement in patient care, training of specialised health professionals, and research for new treatments and better understanding of disease and is world famous for competence in oncological clinical care and for pioneering research directed to understanding of cancer biology.

Located in up-town New York, separated from the East River by the Rockefeller University, it is a complex set of buildings dating from the 1930s. The in-patient hospital, day-care hospital, consultation suites, diagnostic and scanning centres, radiotherapy centre, clinical research and the vast supporting services are located in a rectangular campus enclosed by York and First Avenue running North and South and by 67th and 68th Street East and West. There are also research facilities in the Rockefeller Research Laboratories and the Kettering Laboratories. The Cornell University medical school and the hospital for special surgery are also nearby.

MSKCC had a long and complicated metamorphosis. In 1884, when the Statue of Liberty was unveiled, the cornerstone for the New York Cancer Hospital was also laid. Marion Sims, a gynaecologist with visionary foresight pledged to philanthropists of those days that cancer would be treated by specialised doctors in a designated hospital. Located at the upper West corner of the Central Park, the building in French Gothic style which still stands today was finished in 1887. In 1899, the hospital was renamed the General Memorial Hospital. James Ewing brought scientific research into the hospital in 1913 and James Douglas, a mining engineer who had lost a daughter to cancer, supplied radioactive minerals and the know-how to harness them into clinical use. In 1916, the hospital underwent another name change to the one in use today. In 1927, John D. Rockefeller Jr., funded the first fellowship training

program for specialised doctors in treating cancer and his continued support lead to a major up-grading of the Hospital opened more than half a century ago. The Hospital then moved to its present site on York Avenue at the Upper East Side.

In 1945, to celebrate the end of World War II, three distinguished industrialists at General Motors, Alfred P. Sloan Jr, Charles F. Kettering and Frank A. Howard contributed to establish a formal research institute, the Sloan-Kettering Institute (SKI) in recognition of the importance of academic research in finding new cures for cancer. A crucial difference which sets SKI apart from other research institutes is its close link with clinical practice: most of the laboratory investigators also held clinical posts at the Memorial Hospital, a tradition still found today. The recognition of the need for further integration between treatment, research and education led the Memorial Hospital and SKI to unite and the Memorial Sloan-Kettering Cancer Centre was thus formed. This was followed by a period of expansion based around the site of the Memorial Hospital.

It was the goal of the founding benefactors that the staff of MSKCC should work in the best possible environment. In 1967, a cyclotron was acquired to produce radionuclides for diagnostic and therapeutic purposes. Freshly prepared radioisotopes from these on-site accelerators are often incorporated into modern immuno-therapeutic references in which MSKCC is a world leader. In keeping with their pioneering work in radiotherapy, they are also one of a handful of institutes with 3D conformal radiotherapy facilities.

In 1971 the US government passed the National Cancer Act and nominated MSKCC as the prototype comprehensive cancer centre. The need for more hospital beds were met when the 19 storey 565 bed New Memorial Hospital was opened in 1973. It brought all the clinical investigations and comprehensive care for cancer sufferers under one roof.

Whilst chemotherapy and radiotherapy had undergone steady refinement, with MSKCC staff contributing to new improved treatment protocols, their toxic side-effects remained the limiting factor in their use. Scientists at SKI discovered two of the earlier cytokines, granulocyte colony-stimulating factor (G-CSF), and granulocyte and macrophage colony-stimulating factor (GM-CSF), which exerted powerful stimulatory effects on development of the haemopoietic system. They have revolutionised bone marrow transplantation and lead to the emergence of peripheral stem cell transplantation (PBSC) which dramatically shortens the vulnerable period following bone marrow transplant. PBSC has also produced substantial improvement in treatment of leukaemia and advanced germ cell tumours, and other solid tumours including breast and ovarian may also benefit from this treatment. Towards the end of the 80's the Rockefeller Research Laboratories were built. Potential therapeutic agents prepared in laboratories can now be swiftly delivered to the hospital for clinical testing.

During my stay at the hospital, I observed the comprehensive approach to cancer care. The centre provides facilities ranging from screening for disease to diagnosis, treatment and long-term surveillance, all utilising the best tools available. Patient care is provided by disease management teams of specialists working together including surgeons, physicians, radiologists, pathologists and other staff. Other issues of patient care such as pain and psychological trauma are also managed by specialists. In the current climate of private health care in the USA, only institutions with such a long and outstanding reputation and the tremendous throughput of patients can afford to provide this level of care and fulfil what Sims envisaged to achieve in 1884.

Telomere biology

It was my good fortune to be able to spend twelve weeks learning about molecular biology as related to carcinogenesis and the specific area of research was telomere biology. In 1938, J Hermann, who was working in Professor F. A. E. Crew's department of animal genetics in Edinburgh University, discovered that the genome of the fly *Drosophilia* can be damaged by X-ray irradiation. He reasoned that the chromosomal breakages must reunite in a specific order and that no deranged reunification would occur; he proposed that the free ends of chromosomes have distinctive functions and he suggested that this discrete area contained genes required for the stable inheritance of genetic material from generation to generation; he called these telogenes (telos, end in Greek) and when during metaphase they become visible bodies, telomeres (meros, part in Greek). These free ends were different to the ends of a chromosome when it is split in the middle. Whilst the artificial breakages can spontaneously recombine, the natural free ends of chromosomes do not fuse with each other.

The gene sequence of telomeres was unknown until 1978 when Elizabeth Blackburn sequenced the telomeres in a protozoan; this turned out to be an array of tandem repeats. Subsequent experiments confirmed that most animals contained such tandem repeats at the ends of the genome. In humans, the repeats contain the nucleic base sequence of TTAGGG. It is now accepted that the capping telomere at the ends of a genome protects it from radiation damage and from erroneous recombination of breakages; they are actually added on to the broken ends and so prevent spontaneous recombination.

The enzymatic mechanism proposed for DNA replication had an Achilles heel: as the ends cannot be replicated, as shown by James D. Watson in 1972, this leads to progressive loss of the genome with repeated rounds of replication. The length of telomere repeats fluctuates during *in vitro* culture of cells. In fact the telomere provides a solution for the replication problem. The tandem repeats, which can have up to 2000 copies at one end in humans, allow the DNA to replicate without replicating the ends. As the telomeric sequence become shorter, it is proposed that an enzyme, telomerase, is activated and replaces copies lost to incomplete DNA replication. The precise mechanism for such activation is under much investigation at present.

In the 1960s, Hayflick elegantly demonstrated that human cells *in vitro* have limited potential for replication; at the so called Hayflick limit, cells become senescent with characteristic changes and stop dividing. The progressive shortening of human telomeres with age is an attractive explanation for this biological observation. As the ends shorten and are no longer able to perform their function, the whole cell also become non-viable as its genome is no longer intact. The mechanism for this is complex and is only beginning to be unravelled. Proteins which bind specifically with a telomeric repeat was first isolated in 1995 and are now thought to be as important as the tandem repeats to form a functional telomere unit. This protein/DNA unit is likely to mediate the interaction of genome with the nuclear membrane and other cellular mechanisms with importance in the phenomena associated with the cell cycle and apoptosis.

To extrapolate the Hayflick limit further, cancer cells which appear to be immortal, at least *in vitro*, must have somehow overcome replicative senescence. In 1994 evidence emerged to support the view that telomeres may play a role in cell immortality. Telomerase was present in the majority of cancer cell lines and more impressively in freshly removed tumour material.

Telomerase is a highly regulated enzyme, only protein extracts from normal mammalian germ cell tissues and from the bone marrow contain activity. The tightly regulated activity could be a protective mechanism preventing cells which harbour a damaged genome from proliferating. Cancer cells which have reached the Hayflick limit, but then acquired an immortal phenotype with the concomitant activation of telomerase, have been described.

I worked with the research group lead by Dr. Malcolm Moore, the discoverer of the cytokine G-CSF and who is currently testing drugs which inhibit telomerase. It is envisaged that telomerase inhibitor may be an entirely new class of therapeutic agent active against cancer cells. My task was to develop a sensitive assay for measuring the loss of telomeric repeats in the genome. The current methodology measures all telomeric containing fragments from the digestion of genomic DNA by restriction endonucleases which spare the telomeric sequences. The telomere length is then expressed as a mean or peak value after statistical manipulation. The behaviour of telomeres from different chromosomes within the same cell is unknown at present. The change in length due to cell replication and addition by telomerase may not be uniform across the chromosomal termini. It is also known that small amounts of telomeric sequences are found in the inner parts of a chromosome. Due to these factors, the current assay is not sensitive enough to measure small changes in tandem repeat length and may introduce confounding errors in the analysis hence making the interpretation of results extremely difficult. A more sensitive method is thus needed to measure the precise loss of repeats per cell replication which may be wrongly estimated by the current method. The dynamic changes in repeats length in tissues during a period of rapid proliferation is perhaps more interesting especially during the phase with active telomerase. I managed to lay the ground for an improved assay for measuring the telomeric length at a single end of chromosome. Some preliminary data showed that there are differences in the measurements and the work will be pursued further. It is my hope that with optimised conditions, the selectivity will be able to answer some of the questions posed by current experimental evidence.

It was a privilege to be able to work with such a dynamic and talented group. The Institute also provided plenty of opportunities to learn about other areas of science from their distinguished visiting speakers and equally brilliant staff. I also gained much from the clinical meetings where new research was presented and refinements for present treatments suggested. The sense of urgency which echoed within the Institute was more than a match for the pace of New York.

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