Institution: University College London

Unit of Assessment: 4 - Psychology, Psychiatry and Neuroscience

Title of case study: Development of conditionally immortalised cell lines as novel cell models of disease and for cell transplantation

1. Summary of the impact

Research by Professor Parmjit Jat (first at the Ludwig Institute for Cancer Research, then part of UCL; later at the UCL Institute of Neurology) established and applied the critically important scientific concept of conditional immortalisation to a wide variety of cell lines, enabling cells to be grown indefinitely *in vitro* but differentiate upon altering the growth conditions. Two companies were established in partnership with Jat to exploit this research, ReNeuron (now worth £63.5m and publicly traded on the London AIM market) and XCellSyz (now part of Lonza AG). More than 20 patents based on Professor Jat's work have been issued. Reagents based on his research have been evaluated, licensed and used by 17 companies worldwide: Amgen, Amylin, Boehringer Mannheim, Cell Genesys, Chiron, Eli Lilly, Genentech Inc., Genetics Institute, Immunex, Johnson & Johnson, Medarex, Novartis, Ortho Pharm., Pfizer Inc., Regeneron, ReNeuron, Takeda, EMD Serono, and XCellSyZ/Cambrex Bioscience/Lonza.

2. Underpinning research

Professor Parmjit Jat established the critically important concept of conditional immortalisation, whereby cells can be grown indefinitely but undergo a rapid cessation of growth and differentiate upon altering the growth conditions. This enabled him to develop "Immortomouse", a novel H-2K^{b}tsa58 strain of mice that can be used to derive conditionally immortal cells from a wide variety of tissues. This was patented by the Ludwig Institute for Cancer Research (LIRC) and the Medical Research Council (MRC) with Jat as inventor, and has been sold worldwide by Charles River Laboratories. Many research groups and companies worldwide have subsequently used this approach to develop conditionally immortal cell lines that can undergo differentiation.

Collaborative work between Jat, J Sinden (now Chief Scientific Officer of ReNeuron) and JA Gray (originally Institute of Psychiatry, King's College London) led to the development of a conditionally immortal, multipotent cell line from embryonic day 14 hippocampal neuroepithelium of the H-2K^{b}tsa58 transgenic mouse **[1]**. These cells can be maintained indefinitely and exhibit multipotential differentiation properties *in vitro* and *in vivo*. Moreover these cells selectively repopulated a lesioned CA1 pyramidal layer and restored ischaemia-induced deficits in acquisition of a hidden platform location in the Morris water maze.

These results raised the possibility of successfully developing multipotent clonal cell lines that can be used in human graft neurosurgery with the capacity to migrate to areas of CNS damage and restore specific neurological and cognitive deficits. This was the basis for the establishment of ReNeuron with the primary objective of developing novel human cell lines for transplantation.

The demonstration that SV40 large T antigen cooperates with reconstitution of telomerase activity to immortalise human cells enabled Jat to take an important step forward and develop reagents for the conditional immortalisation of human somatic cells. He showed that human cells are not immortalised upon reconstitution of telomerase activity alone but additional activities are required which can be provided by large T antigen **[2]**. Such immortalised cells remain dependent upon large T antigen to maintain their growth; its inactivation results in a rapid irreversible growth arrest. Jat further showed that large T antigen interacts with Bub1, a spindle assembly checkpoint protein, providing a potential explanation for the chromosome aberrations and aneuploidy often observed in large T antigen immortalised cells. This enabled him to develop a triple mutant of large T antigen that does not interact with Bub1, does not bind to DNA and is thermolabile for preparing minimally immortalised counterparts of primary human cells **[3]**.

These reagents have enabled the development of entirely new human cell models, for example, breast epithelial cells **[4]**, podocytes **[5]** and kidney tubule epithelial cells from Dent disease patients **[6]**.

3. References to the research

[1] Sinden JD, Rashid-Doubell F, Kershaw TR, Nelson A, Chadwick A, Jat PS, Noble MD, Hodges H, Gray JA. Recovery of spatial learning by grafts of a conditionally immortalized hippocampal neuroepithelial cell line into the ischaemia-lesioned hippocampus. Neuroscience. 1997 Dec;81(3):599-608. http://dx.doi.org/10.1016/S0306-4522(97)00330-8

[2] O'Hare MJ, Bond J, Clarke C, Takeuchi Y, Atherton AJ, Berry C, Moody J, Silver ARJ, Davies DC, Alsop AE, Neville AM, Jat PS. Conditional immortalization of freshly isolated human mammary fibroblasts and endothelial cells. Proc Natl Acad Sci USA. 2001 Jan 16;98(2):646-51. http://dx.doi.org/10.1073/pnas.98.2.646

[3] Cotsiki M, Lock RL, Cheng Y, Williams GL, Zhao J, Perera D, Freire R, Entwistle A, Golemis E, Roberts TM, Jat PS, Gjoerup OV. Simian virus 40 large T antigen targets the spindle assembly checkpoint protein Bub1. Proc Natl Acad Sci USA. 2004 Jan 27;101(4):947-52. http://dx.doi.org/10.1073/pnas.0308006100

[4] Docquier F, Kita GX, Farrar D, Jat P, O'Hare M, Chernukhin I, Gretton S, Mandal A, Alldridge L, Klenova E. Decreased poly(ADP-ribosyl)ation of CTCF, a transcription factor, is associated with breast cancer phenotype and cell proliferation. Clin Cancer Res. 2009 Sep 15;15(18):5762-71. http://dx.doi.org/10.1158/1078-0432.CCR-09-0329

[5] Sakairi T, Abe Y, Kajiyama H, Bartlett LD, Howard LV, Jat PS, Kopp JB. Conditionally immortalized human podocyte cell lines established from urine. Am J Physiol Renal Physiol. 2010 Mar;298(3):F557-67. http://dx.doi.org/10.1152/ajprenal.00509.2009.

[6] Gorvin CM, Wilmer MJ, Piret SE, Harding H, van den Heuvel LP, Wrong O, Jat PS, Lippiat JD, Levtchenko EN, Thakker RV. Receptor-mediated endocytosis and endosomal acidification is impaired in proximal tubule epithelial cells of Dent's disease patients. Proc Natl Acad Sci USA. 2013 Apr 23;110(17):7014-9. http://dx.doi.org/10.1073/pnas.1302063110

4. Details of the impact

1. Establishment of ReNeuron

The research has led to the establishment of two companies. **ReNeuron** was established in 1997 by Jat's collaborators with £5m in venture capital from Sir Chris Evans after licensing the IP **[a]**. The initial focus was to develop stem cell lines for generating new neurons and brain cells to reverse damage to brain tissue caused by stroke. Jat was a consultant to ReNeuron from 1999 to 2005. ReNeuron is now a £63.5m company (July 2013 figure), publically traded on the London AIM market. ReNeuron have used their cell expansion and screening technologies to develop stem cell therapies for conditions such as stroke where there are few alternative treatments. Unlike conventional drug treatments that treat symptoms, stem cell treatments treat the underlying cause of the disease. The aim is to develop cells that can be readily administered "off-the-shelf" to any eligible patient without the need for additional drug treatments **[b]**.

ReNeuron's lead therapeutic candidate is the ReN001 stem cell therapy for the treatment of patients left disabled by stroke. ReNeuron are also developing stem cell therapies for other conditions such as critical limb ischaemia, a serious and common side effect of diabetes, and blindness-causing diseases of the retina. Reneuron have also developed a range of stem cell lines for non-therapeutic applications – the *ReNcell*® products for use in academic and commercial research. The *ReNcell®CX* and *ReNcell®VM* neural cell lines are marketed worldwide under license by Merck Millipore (USA).

ReN001 is currently undergoing the PISCES (Pilot Investigation of Stem Cells in Stroke) study. This is the world's first fully regulated clinical trial of a neural stem cell therapy for disabled stroke patients. Stroke is the third largest cause of death and the single largest cause of adult disability in the developed world. The PISCES clinical trial is being conducted in Scotland at the Institute of Neurological Sciences, Southern General Hospital, Greater Glasgow and Clyde NHS Board. It is a Phase I single administration dose escalation safety study, to 12 stroke patients disabled by ischaemic stroke, the most common form of the condition. The Principal Investigator is Professor Keith Muir, SINAPSE Professor of Clinical Imaging, Division of Clinical Neurosciences at the University of Glasgow. The aim of this trial is to evaluate the safety of the implantation procedure and determine the side effects associated with the cell implantation. Patients in the PISCES trial will be followed up over a two year period. Monitoring of the patients will continue for longer term following the two year end-point. In addition to safety and tolerability of ReN001, a number of clinical assessments of the patients will be made to evaluate changes in motor and cognitive function. In June 2012, interim data from the PISCES study for the first five patients was presented by the Glasgow clinical team at the 10th Annual Meeting of the International Society for Stem Cell Research (ISSCR) in Yokohama, Japan **[c]**. Reductions in neurological impairment and spasticity were observed in all five patients compared with their stable pre-treatment baseline performance. Remaining patients in the PISCES trail will be treated by 2013 **[d]**. ReNeuron have already submitted an application to the UK regulatory authority to commence a multi-site Phase II clinical trial to examine the efficacy of ReN001 stem cell therapy in patients disabled by ischaemic stroke. The aim is to recruit patients between two and four months after the stroke.

2. Establishment of XCellSyz

XCellSyz was established in 2005 as a spin-out from UCL by Professor Peter Shepherd; Jat was on the Scientific Advisory Board. XCellSyz was bought by Cambrex and then Lonza AG the current owners **[e]**. Jat, with research support from ReNeuron (1999-2005), developed reagents for conditional immortalisation of human cells. Lonza now supply these reagents as well as conditionally immortal cells to the worldwide scientific community. They describe the benefits of these products as follows: "*Lonza overcomes [the limitations of immortalized cell lines] using a temperature dependent, conditional immortalization approach utilizing Large T-Antigen that allows the immortalization to be reversed and the cells to revert to their original characteristics. This allows development of cell lines from a wide range of human and animal tissues, and of novel models relevant to drug discovery based on lines from people with particular diseases*" **[f]**.

3. Patents

More than 20 patents based on Professor Jat's work have been issued, of which three are currently active **[g]**.

5. Sources to corroborate the impact

[a] Information on the formation of ReNeuron and initial investment available from: http://www.excalibur-group.co.uk/people/executive/prof-sir-chris-evans/stem-cell-research/

[b] Company information on Reneuron is available from: http://www.reneuron.com/ Annual report for and accounts for year ending 31st March 2013: http://www.reneuron.com/images/stories/Financial%20Reports/Reneuron%20Group%20plc%20Annual%20Report%202013.pdf

[c] http://www.reneuron.com/press-release/update-on-stroke-clinical-trial-and-notification-of-interim-results-monday-28-november-2011

[d] Updates on the PISCES trial, May 2013: http://www.lifesciencesscotland.com/connections/news/news-content/update-on-pisces-stem-cell-stroke-trial-encouraging-(1).aspx

http://www.gla.ac.uk/news/headline_279079_en.html

[e] News stories about acquisitions: http://www.genomeweb.com/cambrex-buys-immortalized-cell-line-assets-xcellsyz
http://www.genengnews.com/gen-news-highlights/lonza-makes-its-largest-acquisition-cambrex-bio-businesses-for-460m/7471071/

[f] http://www.lonza.com/products-services/bio-research/primary-and-stem-cells/conditionally-immortalized-cells.aspx

[g] The three currently active patents are:

1. Transgenic mouse cells expressing ts SV40 large T
   Inventors: Parmjit Singh Jat, Dimitris Kioussis, Mark David Noble
   Publication date: Nov 18, 1997
   https://www.google.com/patents/US5688692

2. Transgenic mice expressing TSSV40 large T antigen
   Inventors: Parmjit Singh Jat, Dimitris Kioussis, Mark David Noble
   Publication date: Feb 2, 1999
   https://www.google.com/patents/US5866759

3. Conditional immortalization of cells
   Inventors: Parmjit Jat
   Publication date: Jun 4, 2002
   https://www.google.com/patents/US6399384