JAMES RENNIE BEQUEST

REPORT ON EXPEDITION/PROJECT/CONFERENCE

Expedition/Project/Conference Title: Internship at the Philadelphia Fox Chase Cancer Centre USA .....

Travel Dates: From the 11th of June to the 28th of August 2009 .....

Location: Fox Chase Cancer Centre, Philadelphia, USA .....

Group Member(s): Melissa Lennartz-Walker .....

Aims: Creating iPS cells in the hope of repairing genetic lesions and curing NF1-related plexiform neurofibromas in mice. .....

OUTCOME (not less than 300 words):-

This summer I undertook a summer internship at the Fox Chase Cancer Centre in Philadelphia, USA. Although my general aim was to acquire both theoretical and practical laboratory skills, I was lucky enough to take a very active part, under the supervision of Dr.Chernoff, in the first stages of a very novel and groundbreaking project: creation of iPS cell with the idea of curing NF1-related plexiform neurofibromas in mice.

In human beings, NF1 most often arises as result of germ line transmission of a defective Nf1 allele, followed by somatic loss of the second allele in Schwann cells. Development of neurofibroma is completely dependent on the presence of **heterozygous bone-marrow derived cells**, as only individuals with Nf1+/- bone marrow and Nf1-/- Schwann cells, show unusual Schwann cell growth and fibrosis. With the advent of iPS technology, we have the opportunity to intervene in this process by repairing the defect in the tumour stroma of NF1 heterozygotes, by converting somatic Nf1+/- cells to iPS cells, transforming them, by homologous recombination into Nf1+/+ cells and reinjecting them into the bone marrow of affected mice, after having been converted them to hematopoietic cells.

During the course of the summer, I thus mainly participated in the creation of the iPS cells, and the design of the genetic sequence which would replace the genetic defect, by homologous recombination.

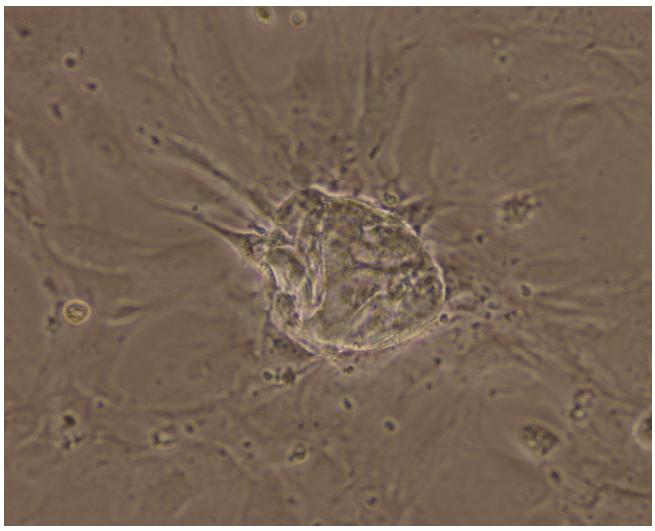
Production of iPS cells requires the transfection of somatic cells with a defined set of genes: c-*Myc*, *Klf4*, *Oct4* and *Sox2*. It had previously been shown that successful expression of these factors by the somatic cells, transforms them into pluripotent cells: iPS cells. In the past,

predominantly lentiviruse vectors were used. We however, used a virus free method, inspired by Kaji et al. 2009, and Woltjen et al. 2009, from which we obtained 2 groups of plasmids, containing the appropriate genes. Transfection was be undertaken for the first set of plasmids and nucleofection was done for the second set of plasmids, and later efficiencies would be compared.

During the month of June my job was thus to order the appropriate plasmids, and the primary NF1+/- cell lines. The plasmids arrived as bacterial stocks, from which the DNA was extracted by Minipreps and checked using a restriction digests. For maximal transfection or nucleofection efficiency, numerous NF1+/- cells were necessary and at a confluence point of 70%, thus the original vial was cultured and passaged 3 times before transfection could take place.

For each technique, 6 wells from 6well- plates, containing different plasmid and buffer DNA were prepared, transfected or nucleofected. 24 hours later the plates had to be fed daily with ES cell media (made in the lab), and checked for iPS colonies. As the plasmids from the Kaji et al. Lab contained an Orange gene, its integration and expression could be tested by flow cytometry, day 12 post transfection. This was undertaken in a special FACS facility. Results from a single well thus gave us an indication of the nucleofection efficiency.

Happily coinciding with my last day of work, day 10-post transfection we spotted the first iPS colonies:



<u>Figure 1:</u> Photograph of our first visible iPS colony

During the cell line preparation and post transfection culturing, I designed the genetic sequence supposed to replace the defective mutant Nf1+/- allele in neurofibromas mice. Having obtained the wild type sequence from a BAC clone, PCR primers, homologous to the sequence to be replaced, were designed. The resulting PCR product was thus the wild type allele flanked by a

homologous sequence to the defective allele and restriction sites at both ends. The later restriction sites enabled it to be inserted into a pKO Scrambler plasmid, ready for future transfection of the iPS cells.

All in all, this internship enabled me to gather a phenomenal amount of experience and knowledge, and also gave me an insight as to how the research environment functions. I met some fantastic people, who gave me the desire and motivation to continue in the scientific direction.

Culturally, this was also an experience. America did appear to me, at the end of this summer, as the Land of opportunity, and whiles I did note many contrasts, both extremes always present, I felt a continuous overhanging optimism emanating from this country, making it very agreeable to work in.

References:

Keisuke Kaji, Katherine Norrby, Agnieszka Paca, Maria Mileikovsky, Paria Mohseni & Knut Woltjen. “Virus-free induction of pluripotency and subsequent excision of reprogramming factors”. *Nature* 2009 ; **458**, 771-775.

Knut Woltjen, Iacovos P. Michael, Paria Mohseni, Ridham Desai, Maria Mileikovsky, Riikka Hämäläinen, Rebecca Cowling, Wei Wang, Pentao Liu, Marina Gertsenstein, Keisuke Kaji, Hoon-Ki Sung & Andras Nagy. “PiggyBac transposition reprograms fibroblasts to induced pluripotent stem cells”. *Nature* 2009; **458**, 766-770.