



Leave it to the master gene

As part of what is billed by the organisers as an amazing list of invited speakers for the 20th year of the Lorne Cancer meeting, Professor Peter Klinken will talk about his group's latest findings on a couple of key decision makers in blood cell differentiation and leukaemia. By Fiona Wylie.

SEVERAL YEARS AGO, Peter Klinken's research team at the Western Australian Institute for Medical Research (WAIMR) in Perth cloned a group of genes that were up-regulated in a rare type of red cell leukaemia called erythroleukaemia. The cells could turn into a myeloid-like or monoblastoid cell line, switching from being immature red blood cells to immature macrophage-like cells and losing all of their erythroid characteristics in the process. Importantly, the switch could be simulated in certain cell lines, called J2E erythroleukaemic cells, *in vitro* under specific culture environments.

Using PCR-based subtractive hybridisation, the researchers isolated genes whose expression was increased or only

present when the cells switch lineage, that is, in cells with the new myeloid phenotype and not in the parental cell line. Further experiments in this erythroid to myeloid lineage switch narrowed the genes of interest down to two, which Klinken called haemopoietic lineage switch 5 and 7 (Hls5 and Hls7).

"We were keen to know how these guys suddenly turned off all their red cell genes and switched on a whole new batch of gene expressions to go down a different cell lineage," Klinken, who has a long-standing research interest in haemopoietic lineage commitment and identifying genes that cause leukaemia, says. This work has taken him into the world of haemopoietic stem cells to identify and understand where

these very early cells are going wrong to end up as leukaemias and what regulates the lineage switch.

As one approach to these questions, Klinken decided to further investigate the genes they had isolated from the chameleon-like leukaemic cells and how these genes were involved mechanistically in the cell differentiation process. "We found that the phenomenon is not all that uncommon in human leukaemias," he says. "Patients present with one form of leukaemia, and then with treatment or sometimes sponta-



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neously, the cancerous cells will suddenly change and develop into a totally different phenotype."

Transcription regulators

The researchers at WAIMR are now concentrating on characterising these two genes, and Klinken will discuss their latest and quite exciting findings in this endeavour at Lorne. The genes turn out to be major transcriptional regulators: one an oncogene and the other a tumour suppressor.

"Hls7 is the murine orthologue of myeloid leukaemia factor 1 (Mlf1) in humans, a putative oncogene involved in acute myeloid leukaemias," Klinken says. "It imposes a monoblastoid phenotype upon the erythroleukaemic cells. Hls5, on the other hand, bears the hallmarks of a novel tumour suppressor gene.

"We now want to know what Hls5 and 7 actually do and how they do it – how do these genes affect the transcription of other genes? They are certainly very important molecules in the transcription process controlling differentiation and lineage commitment. We would therefore like to establish how they affect downstream structural and functional genes."

The work is being carried out mainly in leukaemic cell lines and, more recently, in a number of transgenic and knockout mice generated by the group. Current experiments are focused on identifying the specific molecular targets of Hls5 and Hls7. To this end, Klinken and his team recently published in the journal *Blood* that Hls5 is a negative regulator of transcription factor GATA-1, which is essential for erythropoiesis or red cell lineage commitment.

This finding is significant in elucidating what this gene is doing in a developing

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cell. "If Hls5 is switching the GATA-1 gene off, it is preventing these cells going down the erythroid pathway, leaving them to possibly go backwards or else off towards a myeloid cell line. Therefore, it seems that the Hls5 gene is acting very early in blood cell differentiation."

Klinken's current hypothesis is that Hls 5 and 7 participate in some sort of master switch for haemopoietic lineage determination. "These guys probably act co-operatively in the molecular decision-making associated with erythroid to myeloid cell switching. When things go wonky they are also involved in the resulting leukaemia, and then ultimately in changing phenotypes as well.

"Identifying and studying these genes has provided not only a great tool for understanding what is going on in particular leukaemias, but also, as oncogenes and tumour suppressors, they might ultimately play a critical role in other leukaemias and cancer induction more generally. Ideally, we would like to work out where the pathways regulated by Hls5 and Hls7 have gone wrong and how can we correct it." **ALS**