Retrotransposons, genomic integrity and biosafety

Could you provide a brief overview of your work?

Our objectives are to address the genome destabilising effects of the activity of endogenous transposable elements (TEs), which can affect genomic integrity and host gene expression in human embryonic stem cells (hESCs) and human induced pluripotent stem cell (hiPSC) lines, and to identify factors affecting the activity of TEs. Pluripotent stem cells not only hold great therapeutic promise as cell sources for substitutive and regenerative autologous cell therapies but can also be used for disease modelling, the study of cell development and function and in vitro screening of drug candidates on healthy and diseased cells. We explore whether there are integration preferences of LINE 1 (L1)-mediated retrotransposition events for specific genomic regions in hESCs and hiPSC lines that could potentially affect neighbouring gene expression, and seek to identify host-encoded factors influencing L1 activation and replication in pluripotent stem cells.

We are also investigating the connection between L1 mobilisation and tumour development, mechanisms of intracellular defence against human L1 mobilisation and the composition of L1-ribonucleoprotein (RNP) particles. A mouse model will be used to identify tissues, cell types and developmental stages that support retrotransposition of a marked L1 reporter element in vivo, and for cancer gene discovery.

What excites you about retrotransposon research?

When I was working on my diploma thesis in 1989, there was barely any knowledge about the activity of transposable elements or their impact on structure and organisation of the human genome or the host cell. This prompted many biologists to name TEs ‘irrelevant junk DNA’. Over the last 25 years, tremendous progress has been made; studies have shown that human TEs are not only a major driving force behind evolution with roles in cellular plasticity, but that they can also cause disease through a variety of mechanisms.

How might methylation profiles specific for pluripotent stem cells open the door for activation and retrotransposition of endogenous mobile elements in these cells?

Activation and mobilisation of transposable elements threatens the structure and regulated expression of the genome in several different ways. Once the functional L1 protein machinery is expressed, it can mobilise transcribed Alu and SVA retrotransposons and any mRNA encoded by random host genes in addition to L1 elements. The mobilisation results in de novo insertions of retrotransposon copies into the host genome, which could corrupt genomic integrity and/or affect host gene expression. DNA methylation is considered a host defence mechanism against the mobilisation of endogenous transposable elements, whose activation poses a persistent threat to the integrity of the genome. Transposable elements are highly abundant, rich in CpG dinucleotides and heavily methylated in differentiated cells.

What led you to question the biosafety of hiPSCs and their derivatives?

Since 2011, data have accumulated indicating that the reprogramming process and subsequent cultivation of hiPSCs in vitro can induce genetic and epigenetic abnormalities in these cells. Publications revealed copy number variations (CNVs), protein-coding point mutations and somatic epigenetic memory, as well as aberrant reprogramming of DNA methylation in hiPSCs. These findings questioned whether hiPSCs or their derivatives are safe for administration. Genomic mutations may undermine their use in regenerative medicine.

Finally, several research groups including ours demonstrated that the induced full-length L1 RNA expression observed in hiPSC lines correlated well with an overall decrease in CpG methylation in the L1 promoter region. We show that endogenous L1-mediated mobilisation occurs during the cultivation of hiPSCs and can perturb the expression of key protein-coding genes with unknown consequences in differentiated cells. Taken together, the variety of reported genetic and epigenetic abnormalities raise serious questions regarding biosafety of hiPSCs and their derivatives.

Where do you foresee this line of research heading in the future?

We live in an exciting era for mammalian retrotransposon research. It is becoming increasingly obvious that L1 activity is one cause of genetic mosaicism and genetic disorders. Also, accumulating evidence suggests that L1-mediated retrotransposition events play a role in tumour development. L1 activity can affect genome stability, especially during early embryonic development and in pluripotent stem cells. New technologies are currently being developed to reveal insertions, confirming that interspersed repeat polymorphisms are important sources of genetic variation in human populations, and suggesting that each of us has somatic compartments genetically variegated by insertion events.

One example for such a new technology is the ‘single-cell RC-seq’ method recently developed by Dr Geoffrey Faulkner of the University of Queensland, Australia, which enables the identification of new L1 insertions in single cells. The capacity to locate L1 insertions in individual cells is a major step towards determining whether mosaicism impacts the function of pluripotent stem cells or their derived differentiated cells. We look forward to delineating and altering functions of specific insertions in human disease.

Dr Gerald G Schumann explains the importance of his team’s research on human-specific retrotransposons and the LINE-1 content in human genomes.
Tackling one source of genomic instability

Researchers from the Paul Ehrlich Institut (Federal Institute for Vaccines and Biomedicines), Germany, and other prestigious institutions worldwide are elucidating key processes involved in the destabilisation of the genome. It is anticipated that this research will lead to significant medical advances and health benefits.

The Human Genome is more fluid than we might think. Major sections of the genome have no clear coding function and/or are subjected to changes during replication and reproduction. This unpredictability poses a serious challenge to the development of future stem cell therapies. Embryonic stem cell therapy is a salient and emotive topic, credited with the potential to cure many serious illnesses and criticised with equal fervour for being unethical.

However, a new form of stem cell therapy has emerged in recent years, which involves the use of human induced pluripotent stem cells (hiPSCs). hiPSC-based therapies circumvent many ethical issues and could provide autologous cells compatible with the patient’s immune system. The changeability or plasticity of the genome, however, acts as a hurdle to the development of such therapies.

Challenges and Potential

hiPSCs are derived from cells taken from the adult body (soma), which are then ‘forced’ to express a specific set of introduced genes, causing these cells to reprogramme back to an earlier undifferentiated and pluripotent state. Once reprogrammed, these cells have the ability to proliferate, producing more undifferentiated cells. Crucially, these pluripotent cultures then have the potential to re-differentiate into almost any of the ~210 human cell types derived from any of the three germ layers, thus holding major promise for future medical applications and research.

In this context, hiPSCs appear to be the perfect alternative to the harvesting and use of human embryonic stem cells (hESCs). This new technology is not without its own challenges, however. The complicated process of ‘forcing’ genetic reprogramming in adult cells, and the later cultivation into larger numbers of cells, can cause genetic and epigenetic damage. This damage could undermine the genetic integrity of the cells, rendering them and their differentiated derivatives potentially hazardous for use in medical therapies or research. Of particular concern is the possibility that such cells will undergo uncontrolled mitotic division, forming tumours.

The Evolutionary Importance of Shuffling

Major culprits in the destabilisation of the human genome are the non-long terminal repeat (LTR) retrotransposon families: LINE-1 (L1), Alu and SVA, which are all mobilised by the L1-encoded protein machinery. In some cases, intact L1 elements copy themselves and insert into functional genes, often altering their expression; indeed, L1-mediated retrotrotransposition events in the human genome have been reported to be responsible for 97 disease-producing insertions to date, including haemophilia and cystic fibrosis.

However, the presence of L1 elements in the genome is not completely unwelcome. The action of such elements encourages genomic reshuffling and diversity, and is probably responsible for many human evolution processes. The random shuffling of genetic material and the modification of genes is vital to the evolutionary process – and goes some way to explaining the presence of transposable elements and the like in our genomes.

An improved understanding of mutations that cause damage and disease, with a view to medical advancement of hiPSCs, is therefore crucial. This is the work being undertaken by the Human Transposable Elements Research Group led by Dr Gerald Schumann at the Paul-Ehrlich-Institut (Federal Institute for Vaccines and Biomedicines), Germany.

About 34 per cent of the total human genome is thought to be a consequence of the action of non-LTR retrotransposons, which can become mobilised by the L1-encoded protein machinery, triggering genomic reshuffling. It is the relevance of this process that the group focuses on: “Our research addresses those aspects of the biology of human L1 elements that involve interaction with host-encoded factors, the consequences for genome stability and expression, and the development of human disease,” explains Schumann.

By elucidating the destabilising effects of retrotransposons in both hESC and hiPSC cultures, the team has uncovered a new cause for genomic destabilisation of pluripotent stem cells. Furthermore, the team provides tools to uncover the extent of endogenous L1-mediated mobilisation in a given hiPSC line, thereby improving the reliability of these technologies for use in disease modelling and therapeutic research.

Localisation and Limitations

Some of the most fundamental aspects of this research include the focus on the localisation...
TRANSPOSABLE ELEMENTS

OBJECTIVE
To investigate LINE-1 (L1)-mediated retrotransposition in human pluripotent stem cells, focusing on the consequences for genomic stability and host gene expression.

KEY COLLABORATORS
Geoffrey Faulkner, School of Biomedical Sciences, University of Queensland, Australia
Zoltan Ivics, Division of Medical Biotechnology, Paul-Ehrlich-Institut, Germany
Jose Luis Garcia-Perez, Department of Human DNA Variability, Pfizer-University of Granada and Andalusian Government Center for Genomics and Oncology, Spain
Ulrich Martin, Leibniz-Forschungslabor für Biotechnologie und künstliche Organe, Medizinische Hochschule Hannover, Germany
Zsuzsa Izsvák, Max Delbrück Center for Molecular Medicine, Germany

FUNDING
German Research Foundation (DFG) – Medicine • LOEWE Center for Cell and Gene Therapy Frankfurt/ Main • Clinigene /EC-FP6 – European Commission - 6th Framework Programme

CONTACT
Dr Gerald G Schumann
Head, Human Transposable Elements Research Group
Division of Medical Biotechnology
Paul-Ehrlich-Institut, Federal Institute for Vaccines and Biomedicines
Paul-Ehrlich-Strasse 51-59
D-63225 Langen
Germany
T +49 61 037 73105
E gerald.schumann@pei.de
www.pei.de/gerald-schumann

PROFESSOR GERALD G SCHUMANN obtained a PhD at the Institute for Biochemistry, Friedrich-Alexander University Erlangen-Nürnberg, Germany. He became a postdoctoral fellow under the supervision of Professor Jel O Boeke in the Department of Molecular Biology & Genetics at the Johns Hopkins University School of Medicine, USA. He currently heads a research group, Human Transposable Elements, at Paul-Ehrlich-Institut in the Federal Institute for Vaccines and Biomedicines in Langen, Germany.

The complicated process of ‘forcing’ genetic reprogramming in adult cells and the later cultivation into larger numbers of cells can and does frequently cause genetic and epigenetic damage

Despite the evolutionary importance of L1 genetic reshuffling, the host is capable of balancing these effects – and certain intracellular defence pathways limit the effects of L1 retrotransposon activity. Schumann and his colleagues are attempting to understand how this defence works. Knowledge of the natural process of limitation may allow them to utilise these pathways to prevent genetic instability from arising as a result of the creation of hiPSCs and subsequently increase biosafety of hiPSCs, making them more suitable for use in research and regenerative medicine.

GROUNDBREAKING STUDIES
Armed with cutting-edge techniques and expertise, and with knowledge of the functions surrounding the innate defence pathways that prevent genetic instability caused by L1 activity, Schumann’s group will hopefully be able to address the challenges that arise from producing and culturing hiPSCs as a consequence of L1 activation. If successful, the team will make significant contributions to modern biological research and medicine, providing raw and ubiquitous material with the potential to be differentiated and utilised in a vast range of ways.

Currently, Schumann and his colleagues are making use of host-encoded defence mechanisms against L1-mediated retrotransposition to develop new techniques for the production of hiPSCs, which are intended to reduce the amount of genetic instability caused as a by-product. The hope is that this exciting research will culminate in making hiPSCs safer to use.

INTERNATIONAL ENDEAVOURS

Schumann’s group is collaborating with Dr Geoffrey Faulkner at the Mater Research Institute, University of Queensland, Australia, who developed and provided the applied novel, sensitive high-throughput sequencing technology, and Dr Jose Garcia-Perez at the Pfizer/University of Granada, Spain. Together, they have contributed significantly to our understanding of L1-mediated mobilisation of members of the three families of human specific non-LTR retrotransposons: L1, Alu and SVA in pluripotent stem cells.

They have identified 10 individual, endogenous de novo retrotransposition events that occurred during experimental reprogramming of differentiated somatic cells into iPSCs and one Alu de novo retrotransposition event that occurred during the cultivation of the human embryonic stem cell line H9, indicating that pluripotency supports mobilisation of endogenous transposable elements. Considering the sensitivity of the applied sequencing approach, they estimate that hiPSCs each carried approximately one de novo insertion. Moreover, they have also elucidated the impact that these events have on the genome of the cells, the mutations they induce and their effects on host gene expression.

These mutations themselves are subject to a wide variation of form and function, with L1-mediated retrotransposition affecting individual genomes in many different ways. One form of genetic change that has attracted the group’s attention is chromosomal inversions in the retrotransposon insertion site, as chromosomal abnormalities were observed as a major problem in the cultivation of pluripotent stem cells.