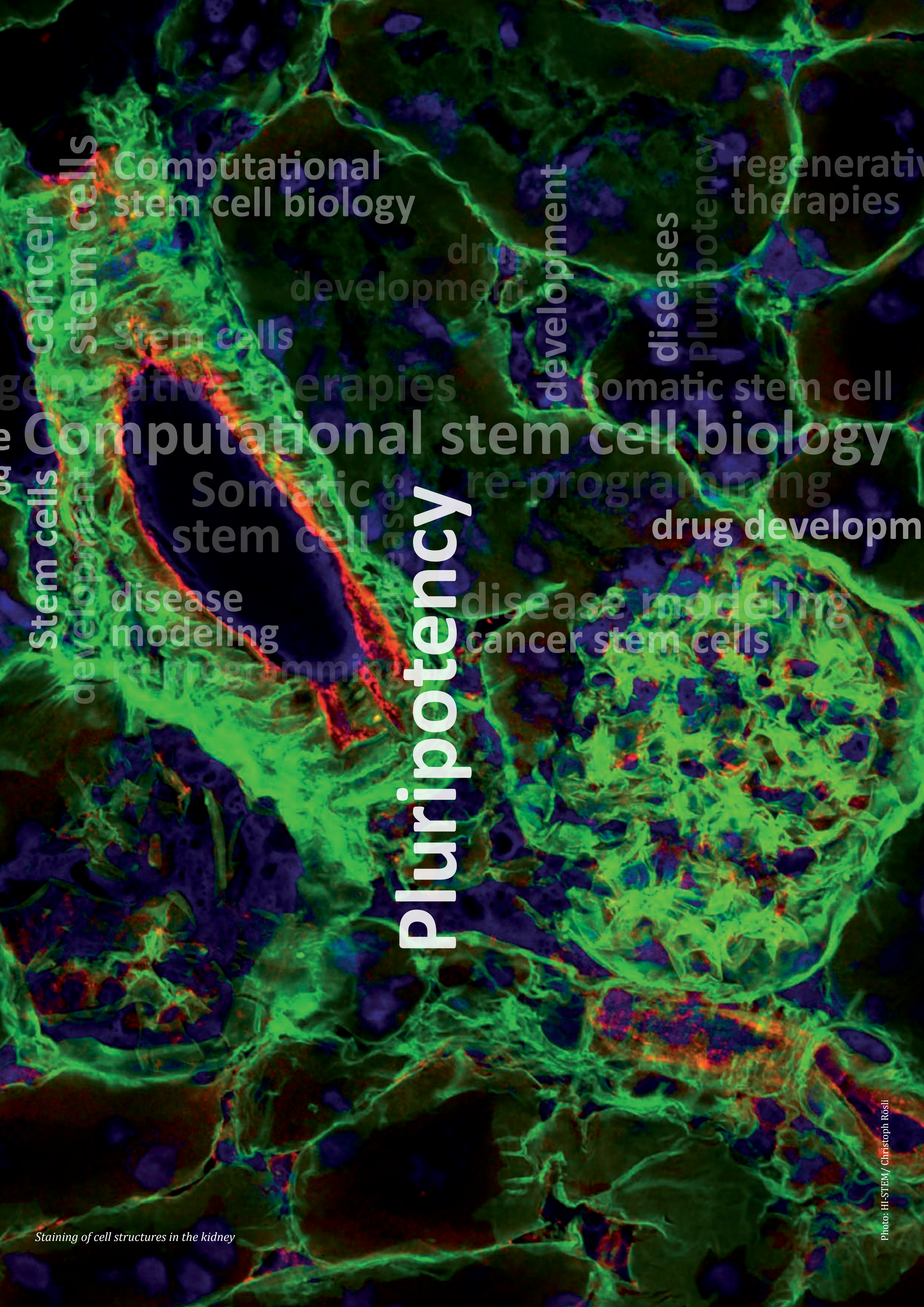


Staining of cell structures in the kidney

Photo: HI-STEM / Christoph Rösli

Pluripotency and re-programming

Flexible cell programs under the spotlight

The field of stem cell research is currently experiencing an unprecedented boom following a series of spectacular advances in cellular reprogramming. Researchers across Germany are unlocking the secrets behind the versatility of cells, and progressively gaining a clearer understanding of the phenomenon of pluripotency. A dedicated GSCN working group has been established to pool outstanding national expertise in this area. The latest molecular biological methods are helping researchers to further the technique of reprogramming and promote translation into biomedical applications.

Shinya Yamanaka first demonstrated the art of reprogramming in mouse cells in 2006 and shortly after also in human cells – an achievement for which he has in the meantime been awarded the Nobel Prize. Since then, new developments in the field have been appearing at almost a monthly rate. Yamanaka's crucial contribution was the celebrated quartet of transcription factors Oct4, Sox2, c-Myc and Kfl4, which are used to convert differentiated skin cells into so-called induced pluripotent stem (iPS) cells (see graphic on page 33). These exhibit very similar properties to embryonic stem (ES) cells, namely, they are able to differentiate into virtually all of the more than 200 cell types of the body, circumventing the ethical issues that have otherwise dominated this area of science. The technology also makes it possible to produce patient-specific cells that will not be rejected by the immune system.

First-class research

Today, German stem cell laboratories are making significant research contributions to the field of pluripotency and reprogramming. This is reflected by a publication analysis published at the end of 2013 by EuroStemCell and the publishing house Elsevier. In a breakdown of the number and citations of publications on the subject of iPS cells in the years 2008 to 2012, two German stem cell research strongholds were in the international top 30: the Max Planck Institute for Molecular Biomedicine in Münster and the Medical School in Hannover (MHH).
Hans Schöler, Director of the Max Planck Institute for Molecular Biomedicine, is one of the initiators of the GSCN working group 'Pluripotency and re-programming'. His research team has been occupied with this and related issues for many years. Among other activities, the Münster-based researchers are studying the special role of the regulator and embryonic stem cell marker Oct4 in development. They are also working towards a more targeted and sparing application of iPS technology. To these ends, the group headed by Holm Zaehres has established the central unit 'Stem Cell Engineering'. The MHH is also regarded as a base for iPS cell production.

The work of Ulrich Martin from the Excellence Cluster REBIRTH, which is also based in Hannover, includes the iPS-based production of cardiomyocytes. Together with his team, he is pressing ahead with the production of large quantities of cells in specially designed bioreactors.

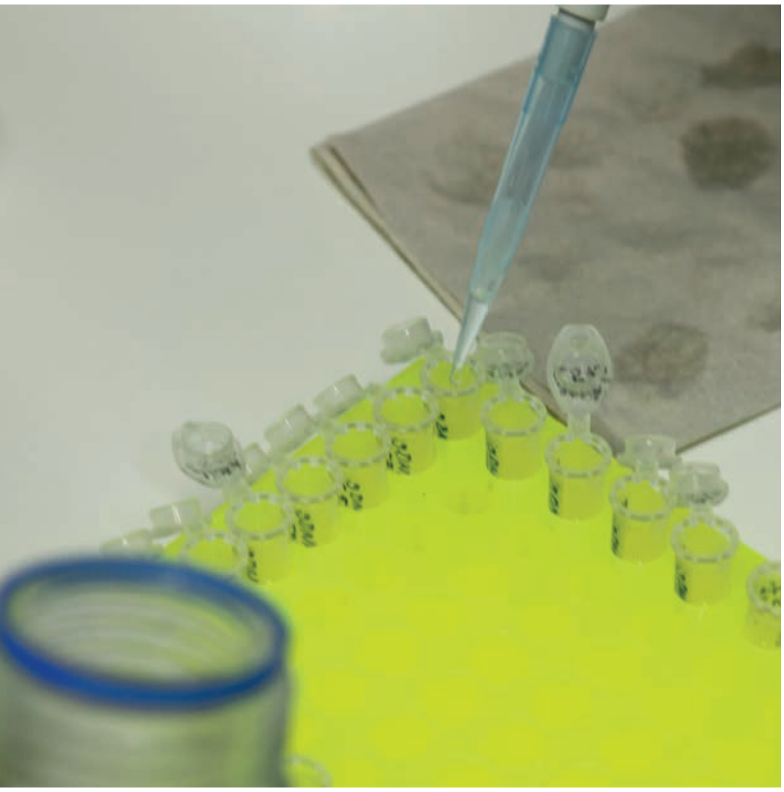
Driving forward iPS technology

The advances that have been made in iPS technology are breathtaking. Researchers in stem cell laboratories in Germany and around the world are continuously refining the formulation and making iPS technology more safe and efficient. Notable is Micha Drukker group among these from the Institute for Stem Cell Research at the Helmholtz Center Munich and the second of the three initiators of the scientific GSCN working group. "Among other techniques, we use artificial mRNA molecules to reprogram the cells," says Drukker. "They quickly and efficiently convert human cells into iPS cells." This means that it is important that production is as speedy as possible, to ensure that further mutations do not accumulate in the cell culture. Drukker is currently setting up a central service unit – the 'hiPS Cell Unit' – at the center's Neuherberg site. On the basis of iPS cells from healthy individuals as well as patients, this will serve as an experimental platform for research partners for the production, thorough verification of iPS cells, and their differentiation into specific cell types. Similar initiatives are underway at other German locations in Berlin, Bonn, Dresden, Hamburg, Hannover, Münster, and span numerous departments at the Bavarian university-based locations of the ForIPS research network.

Understanding complex networks

The artificial induction of pluripotency in cells is one thing, but understanding how this state is actually regulated is another thing altogether, says Drukker. "This happens through a complex interplay of gene expression programs, epigenetic mechanisms and signaling processes," explains the stem cell researcher. "We want to decode the interweaving elements in this ensemble." Drukker is interested in the specific developmental stage in the life of a pluripotent stem cell when it becomes a progenitor cell. The stem cell researchers are now looking for answers for a range of key issues, also in the context of the GSCN working group.

How is pluripotency regulated, in particular in naive cells or other cells that are more restricted in their developmental potential, so-called 'primed' ES cells? What are the differences between ES cells in mice and humans? How similar, in reality, are ES cells and iPS cells? To answer these and other questions, the various cell types and cell stages are being investigated more intense-



Lab impressions

Photo: Life&Brain

ly than ever before. For example, in the laboratories of Martin Zenke and Wolfgang Wagner from the Institute for Biomedical Technologies at RTWH Aachen. And by James Adjaye at the Düsseldorf University Clinic, who is employing systems-biological methods, among others, to approach the phenomenon of pluripotency.

Direct programming

Another development that is the subject of considerable attention is the so-called direct programming or trans-differentiation of one cell type to another, entirely without the intermediate pluripotent stem cell stage. Researchers are now working to further simplify this direct reprogramming of cells. Also here, German researchers are making vital contributions to this emerging field of research. For example, Hans Schöler and his team have succeeded in transforming skin cells directly into neural stem cells. Frank Edenhofer at the University of Würzburg and Oliver Brüstle from the Bonn-based Institute of Reconstructive Neurobiology and LIFE&BRAIN research center are likewise occupied with the direct transformation of skin cells into induced neural stem cells (iNSCs), and with refining the corresponding protocols.

Activating reprogramming in the body

Mathias Treier, research group leader at the Max Delbrück Center for Molecular Medicine (MDC) in Berlin, is the third initiator of the GSCN working group. Taking the perspective of a developmental biologist, he is focused on cellular decisions in living organisms. “One shouldn’t forget that the ES cells and iPS cells generated in the laboratory are artificial systems,” says Treier. For this reason, he considers laboratory-derived cell replacement in regenerative therapies very limited to a few diseases. “Rather, we need to stimulate the self-healing process locally in the organs” says Treier. Being able to identify the right switch is vital in this kind of *in vivo* reprogramming. Treier and his team have discovered a molecular switch in mice that can bring about a functional transformation of ovaries into testicular tissue. Such switching events could also happen in the brain, for example in the transformation of glial cells into neurons. This phenomenon is now being investigated by Magdalena Götz of the Institute of Stem Cell Research at the Helmholtz Center Munich and by Benedikt Berninger at the University of Mainz.

Research networks in Germany

In recent years, various approaches aimed at further exploring the potential of pluripotency and cell programming have been provided with support by the important

Max Delbrück Center for Molecular Medicine (MDC)

MDC MAX-DELBRÜCK-CENTRUM FÜR MOLEKULARE MEDIZIN BERLIN-BUCH IN DER HELMHOLTZ-GEMEINSCHAFT e.V.

Located in Berlin-Buch, the MDC carries out high-quality, interdisciplinary research on basic mechanisms and applications in major human health threats including cancer, cardiovascular and metabolic diseases, and disorders of the nervous sytem. These thematic research areas are supplemented by the Berlin Institute for Medical Systems Biology (BIMSB) at the MDC and the MDC-Charité partnership in the Berlin Institute of Health (BIH).

Interesting projects for PhD students, postdocs, and group leaders are available on themes related to stem cells including hematopoiesis and immune system development, cancer surveillance, cancer stem cells, organ differentiation, etc. Professor Mathias Treier, senior group leader at the MDC, states: “Opportunities for partnerships with clinical groups, a range of cutting-edge technology platforms, and superb animal facilities for diverse model organisms make the MDC an excellent site for stem cell research.”

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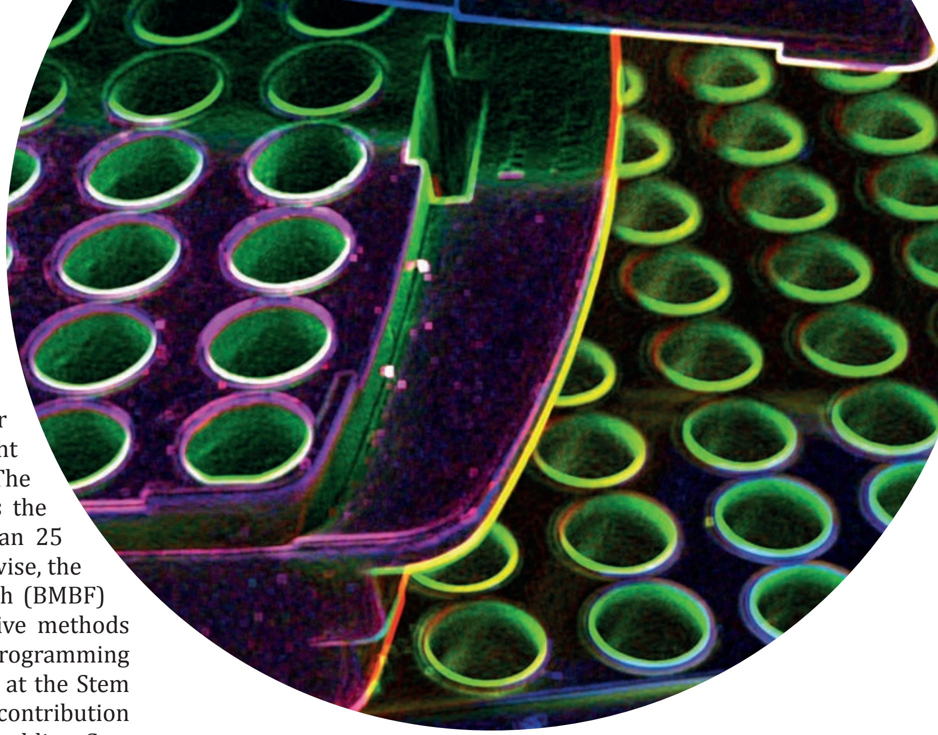

Photo: HI-STEM

German research funding organizations. Among others, the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) has financed the priority program SPP 1356 ‘Pluripotency and Cellular Reprogramming’, coordinated by Albrecht Müller of the University of Würzburg. The program, which began in 2008, comprises the interdisciplinary contributions of more than 25 working groups from across Germany. Likewise, the Federal Ministry of Education and Research (BMBF) is providing targeted support for alternative methods of obtaining stem cells. Projects on cell programming represent a major part of research funding at the Stem Cell Network North Rhine Westphalia. The contribution of these and other research projects are enabling Germany to keep pace with the rapid advances in the field that are taking place globally. However, as result of comparatively restrictive stem cell legislation, the required know-how for the study and use of pluripotent cells has advanced less quickly in Germany than in other countries, where experimentation with human ES cell lines was permitted from very early on.

“We want the GSCN working group to further intensify scientific exchange in Germany,” says Treier. The working group is now set to serve as a nucleus for future collaborations. In the context of the group, the Munich-based researcher Micha Drukker anticipates the development of projects such as online databases, which would be used to share protocols, among other things. The scientist is also considering workshops open to all GSCN members, in which they could pass on their knowledge of new molecular biology technologies.

Text: Philipp Graf