

core facilities
genomics and proteomics
genome editing
iPS core units
model organisms
biobanking
stem cell factories
developmental biology
computational stem cell biology
stem cell imaging
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bioprinting
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computational stem cell biology

Heart muscle cells derived from mouse embryonic stem cells

Measuring stem cells

Deciphering the stem cell code

Thanks to highly sensitive measuring instruments, researchers are learning more and more about the special characteristics of stem cells. High-performance sorting procedures can help to isolate specific cell types. High-throughput analytical techniques make it possible to decipher the molecular signatures of stem cells. The interplay among biomolecules can now be tracked even in single cells. The stem cell as a system can be analyzed more precisely than ever before.

The question of which internal and external factors give a stem cell its special characteristics is one of the key questions being addressed by stem cell researchers around the world. However, decoding stem cells' molecular signatures has proven to be quite a challenge. After all, phenomena like self-renewal or differentiation are based on very complex, dynamic biological programs. Moreover, stem cells, whether pluripotent stem cells or tissue stem cells, take many different shapes.

Luckily, increasingly sensitive analytical techniques are providing stem cell researchers with tools that enable them to constantly improve their analyses of the objects they are studying. Such instruments are the focus of the strategic working group on "Stem cell technologies" of the German Stem Cell Network (GSCN), headed by Frank Emmrich of Leipzig University and Andreas Bosio, Head of R&D in the stem cell division of Miltenyi Biotec GmbH in Bergisch Glad-

bach. "In our group, we want to discuss the wide range of new techniques and promote dialogue about them," says Bosio. He says another goal is influencing and even helping to determine the future development of important cell technologies.

Bringing order into the cell mix

Anyone wishing to take a closer look at specific stem cell types needs samples that are concentrated and as pure as possible. "The basic problem in working with tissue stem cells is that we are dealing with heterogeneous cell populations," says blood stem cell specialist Michael Rieger of the LOEWE Center for Cell and Gene Therapy at the University Hospital in Frankfurt am Main. Furthermore, these cells are rare. In the bone marrow, there is one hematopoietic stem cell (HSC) for every 100,000 cells.

Cell sorting procedures are used to bring order to this mix of cells. In flow cytometry, cells flow rapidly past optical or electrical detectors. They are sorted on the basis of characteristic molecules on the cell surface called markers. Fluorescence-activated cell sorting, FACS for short, has become indispensable for research labs. Miltenyi is banking on magnetic cell sorting, which has been widely used for many years. In that process, magnetic nanoparticles are conjugated with antibodies that dock selectively at markers on



Analysis of FACS data in the laboratory

stem cell surfaces. The main advantage is that by using a magnet, it is possible to fish out the desired cells from large quantities of cells very gently and under sterile conditions. The technique is already used routinely in clinics.

A new cell sorting system from Bergisch Gladbach, on the other hand, is based on micro-fluidics. "We developed a micro-chip with tiny channels and switches that can, on the basis of fluorescence markers, sort cells that are flowing through even more quickly and more gently than is the case with conventional flow cytometers," Bosio explains. This is also possible under sterile conditions, which constitutes a clear advantage for possible use in regenerative therapies.

Omics technologies

Using omics technologies, molecular biologists can look at cells with an unprecedented degree of precision. Omics technologies are high-throughput bioanalytical techniques that permit parallel, comprehensive testing of biomolecules from a biological sample using a largely automated process and in a relatively short period of time.

The speediest development without a doubt has been in DNA sequencing. Breakthroughs in microsystems technology and nanotechnology have accelerated DNA decoding by a factor of millions and correspondingly reduced the costs.

"In recent years, omics techniques have become so sensitive that you can work with very little cell material," says Michael Rieger, a member of the extended board of the

German Stem Cell Network (GSCN). He sees the most recent technological progress in transcriptome analysis as especially relevant. This involves using a transcribed RNA molecule to measure the activity level of genes in defined cell types. Until recently, transcripts were read using DNA microarrays. "But now RNA sequencing is on the rise," says Rieger. With the RNA-seq technique, the identity and quantity of RNA molecules that are present can be determined very precisely.

Exploring the RNA universe

Another benefit is that this technique opens up a completely new view of the cell's RNA universe, which has scarcely been explored. This also makes it possible to investigate functional classes of RNA molecules that are not translated into a protein. These include short microRNAs and long non-coding RNAs (lncRNAs). More and more researchers are now working to determine what role these molecules play in stem cells.

Epigenetic changes, or chemical modifications of DNA, make for another level of genetic regulation. Modern sequencing techniques have made it possible to optimize epigenome analyses, such as studies of DNA methylation patterns. Recording the complete repertoire of protein molecules in a cell (proteome analysis) is likewise indispensable for gaining a precise understanding of biomolecular processes. Thanks to the development of highly sensitive mass spectrometry (MS) techniques, even proteins that are present only as tiny traces can now be identified and even quantified.



Photo: MDC / Himsel

Heidelberg Institute for Stem Cell Technology and Experimental Medicine

HI-STEM gGmbH



HI-STEM

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AND EXPERIMENTAL MEDICINE

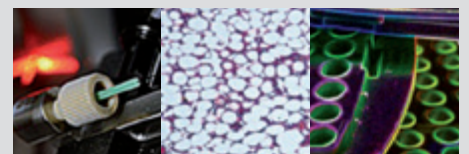
HI-STEM gGmbH is a non-profit public-private partnership between the German Cancer Research Center (DKFZ) and the Dietmar Hopp Foundation (DHS).

Located within the DKFZ in Heidelberg, HI-STEM performs cutting-edge research on stem cells with the aim of translating these results into novel clinical applications. This includes the development of novel diagnostic tools and innovative therapies to monitor and target leukemic and solid tumor stem cells as well as metastatic disease.

Professor Dr. Andreas Trumpp and four Junior Group Leaders direct an international research team of more than fifty employees.

The HI-STEM Research Groups:

- Hematopoietic and Leukemic Stem Cells (A. Trumpp)
- Experimental Hematology (M. Milsom)
- Stress induced activation of HSCs (M. Essers)
- Cancer Stem Cells and Metastasis (A. Trumpp & M. Spick)
- Metastatic Niches (T. Oskarsson)



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Scrutinizing blood stem cells

Scientists in Heidelberg, at the German Cancer Research Center (DKFZ) and the European Molecular Biology Laboratory (EMBL), recently published a study in the journal *Cell Stem Cell* that documents the intensive use stem cell researchers in Germany have already made of omics technologies. In this study, coordinated by Andreas Trumpp, senior president 2015 of the GSCN, hematopoietic stem cells (HSCs) and precursor cells were analyzed molecularly in unprecedented detail. The researchers closely examined the transcriptome, the proteome and the methylome of the various cell types. The combination analysis brought to light about 500 transcription factors and about 700 lncRNAs that show varying patterns of activity in the cell types examined. Michael Rieger, who was not involved in the study, considers the Heidelberg publication a valuable data resource for all blood stem cell researchers around the world. "Now we need to make biological sense of the multiple levels of this information and develop hypotheses from them."

3D organization of the genome

How does the molecular program of a stem cell work? Molecular biologist Frank Buchholz and his team at TU Dresden's medical faculty are using genome-wide functional screens to hunt for important molecular actors that are necessary for stem cell maintenance. The Dresden-based researchers are using RNA interference technology to systematically shut off the genes in cells and test the effects of this intervention. Designer nucleases, the new precision tools of genetic engineering, are also inspiring genome researchers (see chapter on Genome Editing, page 32). "It is also becoming more and more important to consider the spatial organization of the genome in cells," says Buchholz. Buchholz counts the chromosome conformation capture method as one of the hottest topics in molecular cell biology.

Single-cell analysis becoming possible

The omics technologies have become so powerful and sensitive that analysis is even possible at the level of individual

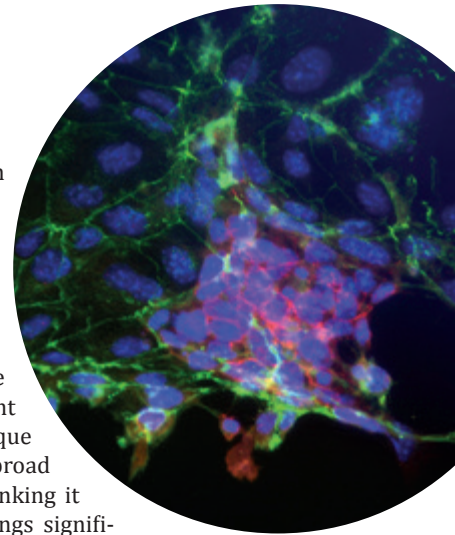
cells. Stem cell researchers can benefit enormously from such single-cell analyses. Andreas Bosio sees great potential in this technique, especially for cancer research. In the study of extremely rare cancer stem cells, for example, testing single cells can provide valuable insight into tumorigenesis. "The technique is still quite cumbersome for broad application," says Bosio, "but linking it with cell sorting can make things significantly easier."

Tracking the fate of a cell

For Frankfurt-based researcher Michael Rieger, however, examining the molecular profile of cells is not enough. To understand how stem cells work, he says, it is also necessary to take a close look at their external form and their behavior. For this purpose, in recent years he has developed a single-cell tracking system in cooperation with Timm Schroeder, who now conducts research at ETH Zurich in Basel. "This system makes it possible to follow the fate of stem cells over long periods of time with a video microscope." Using time-lapse epifluorescence microscopy, individual cells are photographed continuously in a Petri dish. If the cell divides, the tracking software assigns numbers to the daughter cells and then follows their trajectories in real time. Essentially, this constitutes a biopic of the stem cell. "We know about their relationships, their locations, the circumstances of the neighborhood, and the degree of differentiation of each cell," says Rieger. Therefore, he says, the system is ideally suited for studying the influence of external factors on stem cell behavior.

The arsenal of analytical instruments keeps getting more powerful, and the information that is available keeps getting denser. Thus German researchers are getting closer and closer to their goal of deciphering the complex code that makes stem cells so special.

Text: Philipp Graf



Fraunhofer Research Institution for Marine Biotechnology EMB

Innovative research & development at the new institute building of the Fraunhofer EMB in Lübeck



On the 15th of December 2014 the Fraunhofer EMB has moved to the new institute building on the Lübeck BioMedTec Science Campus. The newly constructed building has got modernly equipped laboratories, aquaculture facilities, a food technology center and biobanks with fully automated state-of-the-art technology covering a total area of 8 292 m² (BGF). The technical re-equipment includes a X-ray microscope, a non-invasive small animal MRI as well as several 3D printer of the latest generation, which are used for the development of novel laboratory appliances.

"With these excellent research capacities we look forward to strengthening the life science expertise of the Fraunhofer-Gesellschaft. The new research building gives us the opportunity to explore promising topics for applied research and to promote existing business areas with the most modern equipment" concludes Prof. Charli Kruse, director of the Fraunhofer EMB.

The Fraunhofer EMB works on industry-related research topics with focus on life sciences. Here, novel technologies, procedures and instruments for cell isolation and exploitation were developed. Moreover, the scientists from the EMB work on innovative

aquaculture systems and on the utilization of aquatic raw materials for food engineering. With the "Cryo-Brehm" the EMB maintains one of the largest archives for cell cultures from wild animals.

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