THE DANISH STEM CELL LANDSCAPE

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The stem cell field has attracted a lot of attention in the past years. Exciting new research results are frequently published in the media raising the hope for patients, professionals and the public that one day, stem cells can successfully be used to cure life-threatening diseases such as Diabetes and Alzheimer's disease. This fast growing global attention on stem cell research is also reflected in the Danish research environment. Nationwide, established research groups have expanded their stem cell activities and ventured into new fields such as material science and bioinformatics. New initiatives have furthermore been launched such as the national stem cell centers, DanStem and BrainStem.

This boost in basic research has attracted highly skilled researchers from abroad who have chosen to set up their own research groups in Denmark. Moreover, it has spawned an increased interest from the industry. Several biomedical and pharmaceutical companies have now started to develop and utilize humanized disease models based on patient-derived stem cells for discovery of new pharmaceuticals. There is no doubt that this exciting field will continue to develop rapidly over the coming years and with this article, we hope to be able to give an overview of the various research groups working in the Danish stem cell field.

STEM CELLS

The human body consists of more than 200 different cell types, all of which are carrying out highly specialized functions relative to the tissue they populate. They are the body's smallest living component and a shared feature is that they all arise from a common cell type: The stem cell. Stem cells are the body's ultimate manufacturer of cellular building blocks and are able to repair damaged tissues or organs, a feature, which makes them highly attractive for regenerative medicine.

Stem cells may be found in the early embryo (embryonic stem cells) or in tissues and organs in young and adult individuals (adult stem cells), for example in skin, blood or nervous and muscle tissues. Stem cells are defined by their capacity to self -renew and through this maintain the pool of stem cells while giving rise to daughter cells specified for differentiation.

Pluripotent stem cells. Embryonic stem cells (ESCs) are derived from surplus fertilized eggs from in vitro fertilization treatments. ESCs have the potential to develop into any of the body's more than 200 different cell types, and are thus termed pluripotent stem cells. They are characterized by practically unlimited growth in the undifferentiated stage, given the right culture conditions. Another way of establishing a pluripotent stem cell line is by induction of pluripotency in an adult cell by enforced expression of a number of pluripotency genes. This type of stem cells is termed induced pluripotent stem cells (iPSCs). The potential for developing human disease models for drug testing, and for generating patient-specific cells for transplantation therapies holds enormous promise for pluripotent stem cell research. To date, more than a thousand different ESC lines have been established, and the first European Bank for iPSCs (EBiSC) was established in 2014.

Adult stem cells. For decades, stem cells have been discovered within the majority of our tissues and organs, often clustered in small niches. Most often, the tissue of origin determines the nature of these adult stem cells, and one well-known example is the bone marrow that contains hematopoietic stem cells (HSCs), the predecessors for all different cellular components of blood. Another subtype is mesenchymal stem cells (MSCs), which give rise to bone and cartilage. With successful isolation, these adult stem cells are promising therapeutic tools for regenerative therapies. Moreover, using adult stem cells, scientists are trying to mimic normal human development of different organs, which will lift our understanding of basic human development and disease mechanisms to another level.

A HISTORICAL PERSPECTIVE OF STEM CELLS

The term "stem cell" was introduced for more than 100 years ago by the Russian histologist Alexander Maksimov. In the early 1960s, McCulloch and coworkers demonstrated self-renewing cells in mouse bone marrow, and a lot of scientific work was undertaken throughout the world related to stem cells. A revolution in medical science came in 1968 with the first successful bone marrow transplant between siblings, and ten years later hematopoietic stem cells (HSCs) were discovered in human umbilical cord blood. Another major breakthrough came in 1981 when embryonic stem cells (ESCs) were derived from the inner cell mass (ICM) of mouse blastocysts in simultaneous studies by Martin J. Evans and Gail Martin, who coined the term ESC to these cells. The Nobel Prize in Physiology or Medicine for 2007 was awarded jointly to Mario R. Capecchi, Martin J. Evans and Oliver Smithies for "their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells". http://www.nobelprize.org/nobel_prizes/medicine/laureates/2007/ Figure 1

In 1997, evidence of cancer stem cells (CSC) were presented with leukemia as an example of a stem cell derived cancer. A year later, in 1998, James Thomson and coworkers derived the first human embryonic stem cell line from the ICM of human blastocysts. This work

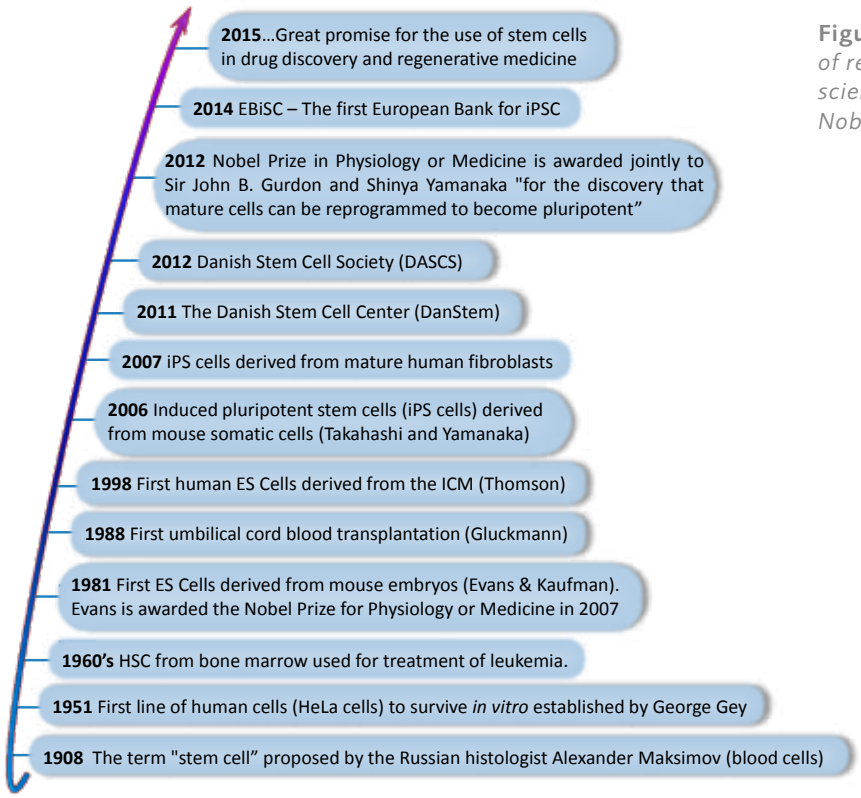

Figure 1. *Time-line for 100 years of research on stem cells with scientific breakthroughs and two Nobel prizes in 2007 and 2012.*

promised great potential for establishing stem cell based therapies, and much effort has since been put into work with ESCs. However, due to ethical considerations and technically difficulties when working with ESCs, other avenues were continuously visited. Hence, in 2006, Japanese researchers Takahashi and Yamanaka published a report of induced pluripotent stem cells (iPSCs) from adult mouse fibroblasts. The Nobel Prize in Physiology or Medicine 2012 was awarded jointly to Sir John B. Gurdon and Shinya Yamanaka "for the discovery that mature cells can be reprogrammed to become pluripotent". http://www.nobelprize.org/nobel_prizes/medicine/laureates/2012/

In parallel with pluripotent stem cell research, several leaps were made in work with adult stem cells. These cells are clinically and therapeutic a subject of intense investigations due to their great potential for replacement therapies. Continuous work within all fields of stem cell research has led to establishment of numerous stem cells based laboratories and research centers at universities, hospitals and in the private sector throughout all parts of Denmark.

DANISH STEM CELL RESEARCH CENTERS

In Denmark, stem cell research is rooted in both basic and clinical research and collaboration across these two fields has recently been strengthened. Danish stem cell research is a broad national anchored endeavour with active research groups in Jutland, Funen and the Capital Region. The individual research groups are working on different stem cell types, from pluripotent stem cells (ESC and iPSC) to adult stem cells (e.g. HSC and MSC). Their individual focus is also different; a number of research groups are working on a deeper understanding of stem cells and their early development from an immature cell to a specialized functional cell type, while others examine basic central regulatory mechanisms linked to major diseases like cancer. As the basic understanding has increased in recent years, a number of clinical focused groups have also been established – in particular within heart disease and orthopaedics. Stem cell research also involves other scientific disciplines, including engineering (e.g. biomaterials) and bioinformatics. The combination of biomaterials with stem cells is for example the starting point for the clinical stem cell research in orthopaedic surgery. Stem cell research, both basic and clinical, is important for Denmark and Danish research groups are increasingly active in the field. In 2012, the Danish Stem Cell Society (DASCS) was established as an independent network for stem cell researchers in Denmark. Here we have summarized information from twelve stem cell research groups in Denmark.

Danish Stem Cell Center (DanStem), University of Copenhagen

The Danish Stem Cell Center (DanStem), headed by Professor Henrik Semb was established in late 2011 at the Faculty of Health and Medical Sciences, University of Copenhagen. DanStem is composed of two sections; a section for basic research in development and stem cell biology (BasicStem) and a section for translational research (TransStem). BasicStem explores fundamental mechanisms governing the ontogeny, renewal and differentiation of stem and/or progenitor cells in both development and in adult homeostasis. They also study how these processes become disturbed in disease states and TransStem facilitates the translation of such findings into clinical practice. Their research covers basal mechanisms behind various cancer types, basal regulatory elements of pancreatic development, and translational aspects with the use of stem cells for diabetes. *Contact: semb@sund.ku.dk*

Biotech Research and Innovation Centre (BRIC), University of Copenhagen

BRIC, headed by Professor Kristian Helin, was established in 2003 as an Elite Centre in Biomedical Sciences by the Ministry of Science, Technology and

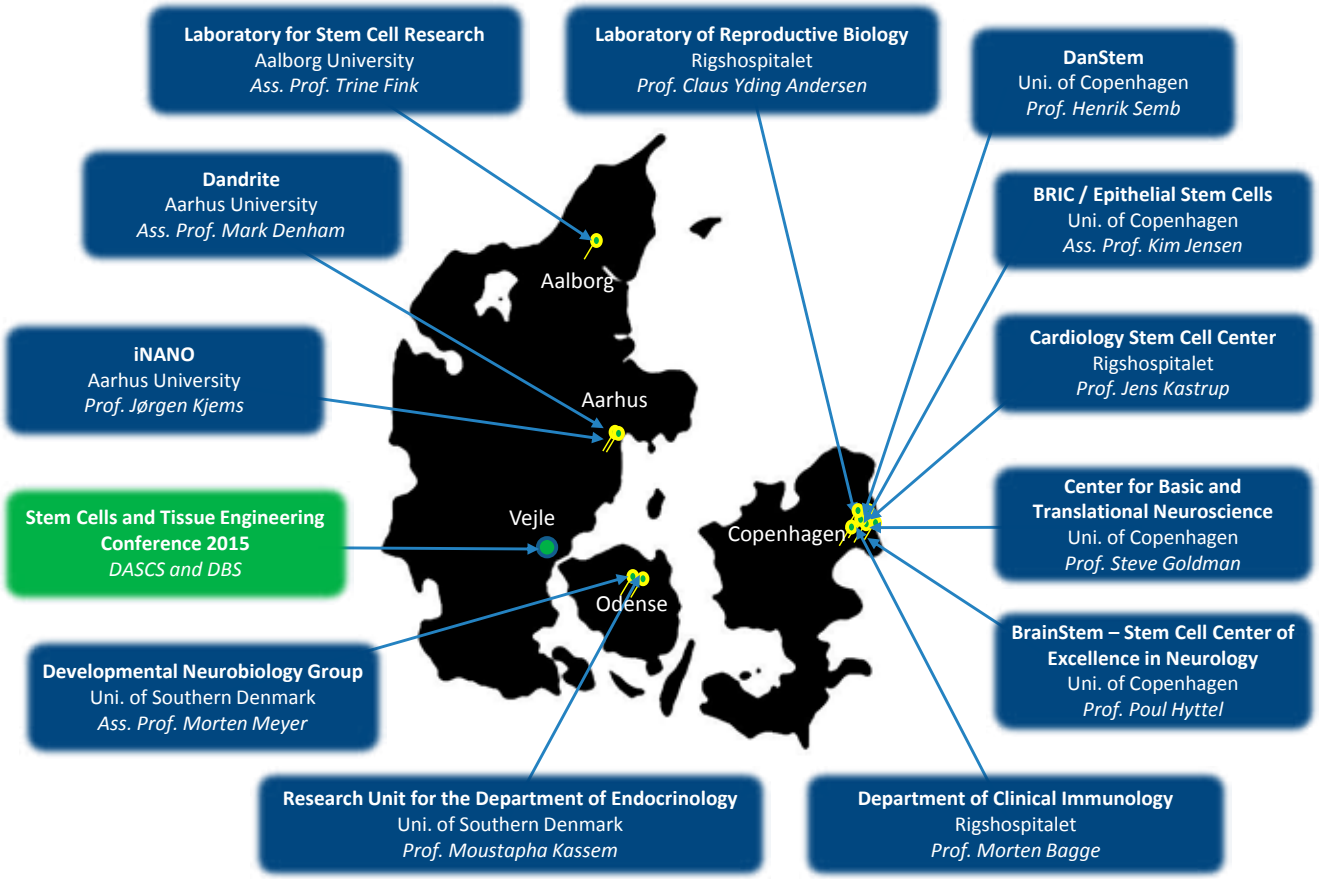

Figure 2. *Danish Stem Cell Landscape. Twelve stem cell research centers located in four Universities: Copenhagen, Southern Denmark, Aarhus and Aalborg. In 2015 the annual meeting of Danish Stem Cell Research Society takes place at Hotel Munkebjerg, Vejle June 4-5.*

Innovation. Here, Dr. Kim Jensen transferred his Cambridge research group in 2013 to continue his research aim of understanding the physiological role of somatic stem cells in tissue homeostasis and characterise molecular mechanisms that control normal and cancer stem cell behaviour. The group is studying tissues such as the skin epidermis and the gastrointestinal tract, which are tissues that demand a continuous renewal. The degree of stem cell contribution to tissue replenishment depends on the specific tissue requirements and is regulated by intrinsic and extrinsic factors including the immediate microenvironment (the stem cell niche).
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BrainStem – Stem Cell Center of Excellence in Neurology, University of Copenhagen
The Brain Stem Center was founded in 2014 and is headed by Professor Poul Hyttel. The vision of BrainStem is to establish a Stem Cell Center of Excellence in neurology across academia and industry with a strong platform for iPSCs as a central core. BrainStem's overall goal is to conduct ground-breaking stem cell research with relevance for neurodegenerative diseases, particularly Alzheimer's disease (AD), frontotemporal dementia (FTD), and Parkinson's disease (PD) based on current ethical guidelines. BrainStem has a strong translational goal: The research activities begin with patients and aim at ultimately benefitting the patients. The overall hypothesis of BrainStem is that patient-specific iPSC lines and their subsequent neural differentiation can provide unique in vitro and in vivo models recapitulating the patient's disease. BrainStem will also address ethical issues related to informed consent and the use of patient-derived stem cells in research and drug development.
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Developmental Neurobiology Group, University of Southern Denmark
The Developmental Neurobiology Group at University of Southern Denmark, headed by Associate Professor Morten Meyer, is focused on identifying molecular and biochemical signals regulating cell fate decisions. Using stem cells derived from developing human foetal brain tissue (neural stem cells, NSCs) and reprogrammed skin cells from patients with Parkinson's disease (iPSCs), the group aim at generating and molecularly and functionally characterizing dopaminergic neurons with midbrain identity – the cell type that degenerates in Parkinson's disease. The overall research aims are to understand the molecular pathology in Parkinson's disease and contribute to the development of new and better treatment strategies.
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Laboratory of Reproductive Biology, Rigshospitalet
The Laboratory of Reproductive Biology at Rigshospitalet, headed by Professor Claus Yding Andersen, have performed research with human embryonic stem cells (hESCs) since the Danish legislation in 2003 allowed the formation of

human ESC lines from surplus embryos, obtained from treatment of infertility by in vitro fertilization. Up to this point, they have established 25 unique stem cell lines. The group uses these cells for both basic research and more clinically oriented studies. In addition, the group has established a method for differentiating ESCs into cardiomyocytes.
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Research Unit for the Department of Endocrinology, University of Southern Denmark
Headed by Professor Moustapha Kassem, the Research Unit for the Department of Endocrinology (KMEB) was established in 2001. The group primarily focuses on basal and clinical research on MSCs with focus on MSC differentiation into osteoblasts and bone formation. The group has been involved in several clinical trials both in Denmark and abroad. KMEB was coordinator of the first clinical stem cell trial in Denmark for vascular regeneration.
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Cardiology Stem Cell Center, Rigshospitalet
Cardiology Stem Cell Center, headed by Professor Jens Kastrup, focuses on clinical stem cell treatment in patients with coronary artery disease, with and without heart failure. The center is approved by the Danish Health Authority for the production of stem cell products as a drug for clinical treatments. They have completed several phase 1 and phase 2 clinical studies of culture expanded MSCs from patients' own bone marrow or adipose tissue. The studies have shown a beneficial effect on patients who gained a better pumping function of the heart and a higher level of physical activity, with fewer symptoms. The group also studies MSCs for treatment of autoimmune diseases and rejection in organ transplants.
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Center for Basic and Translational Neuroscience, University of Copenhagen
Recently (2014), the Novo Nordisk Foundation has supported the establishment of the Center for Basic and Translational Neuroscience at the Faculty of Health and Medical Sciences, University of Copenhagen. The center will be headed by Professor Steven A. Goldman and Professor Maiken Nedergaard, who are both internationally acknowledged for their contribution to neuroscience research. The center will continue their work on a broad set of stem and progenitor cell focused technologies that have permitted them to make substantial progress on many aspects within neurobiology, neuronal disease mechanisms and of neuronal diseases.
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The interdisciplinary Nanoscience Center (iNANO), Aarhus University
iNANO, headed by Professor Jørgen Kjems, was established in 2002. The center commands a variety of facilities for the synthesis of nanostructured/nanopatterned 0D (i.e. nanoparticle), 1D, 2D and 3D materials. The main focus is the interplay between stem cell biology and biomaterials. iNANO has a well-developed infrastructure for synthesis (e.g. electrospinning, 3D printing) and nano-characterization of bioactive materials. The research areas are the influence of topographical and mechanical properties of materials on stem cell development, incorporation of bioactive molecules, like siRNA, microRNA and protein, and their effects on stem cell differentiation, as well as tracking of cells and nanoparticles in biomaterial and whole animals.
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The stem cell laboratory at DANDRITE, Aarhus University
The stem cell laboratory at DANDRITE, headed by Mark Denham, is focused on how the human nervous system develops and the processes involved in neurodegeneration, based on studies of iPSCs. One of the main interests is Parkinson's disease and the group has made stem cells from patients that carry Parkinson mutations. The group has developed specific methods for generating particular dopaminergic neurons that are affected in Parkinson's disease. Using this approach, neurons with a PD mutation can be studied to understand how Parkinson's disease occurs and the specific conditions that may accelerate or slow the disease state.
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Laboratory for Stem Cell Research, Aalborg University
The Laboratory for Stem Cell Research, headed by Professor Vladimir Zachar and Associate professor Trine Fink, is focusing on different types of stem cells, including ESCs, limbal stem cells, corneas, muscle stem cells and MSCs. A specific focus is the use of MSCs for wound healing. It is not yet known why the stem cells have this effect on chronic wounds, but their ability to promote the formation of blood vessels and their ability to inhibit inflammation may play a role. The focus of the research group is clinical trials to demonstrate the effect of MSCs on healing of chronic wounds, and to identify the molecular mechanisms behind the clinical efficacy.
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Department of Clinical Immunology, Rigshospitalet
Department of Clinical Immunology, headed by Medical Director Morten Bagge is specialized in immunology, transplantation and transfusion medicine and includes a Tissue Center and Cell Therapy Facility, headed by Chief Physician Anne Fischer-Nielsen. The main focus areas are the basic characterization and clinical use of HSCs and MSCs from bone marrow and adipose tissue. The department is the main center for HSC transplantations to patients and is constantly investigating stem cell biology related to clinical efficiency of these stem cell treatments. The center is approved by the Danish Health and Medicines Authority for the manufacture of stem cells as Human Medicinal Products.
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STEM CELL RESEARCH IN THE PRIVATE SECTOR

Private companies and organizations in Denmark are also involved in stem cell research and development. Novo Nordisk has for many years been focusing on both basic and translational research within the diabetes area. The stem cell group at Novo Nordisk has a long history in understanding and controlling the development of pluripotent stem cells to insulin-producing beta cells. Lundbeck has recently started to use human iPSCs from neurodegenerative patients to improve their in vitro model systems and to understand neuronal diseases even better. Lundbeck is also involved in the recently established stem cell center, BrainStem. Bioneer is developing stem cell technology plat-

forms, including stem cell production, characterization, differentiation and gene editing, with a primary focus on iPSC technology. Bioneer is also part of BrainStem and of EBiSC.

Danish Stem Cell Society
The Danish Stem Cell Society (DASCS) is a volunteer-driven organization, formed in 2012 at the initiative of three Danish researchers from industry and academia. The motivation was a lack of a national focal point for the Danish stem cell community and an independent forum where stem cell researchers could exchange experiences and establish collaborations across universities, industry and hospitals in Denmark. Currently DASCS has over 300 members and organizes annual stem cell conferences in Denmark. DASCS seeks to strengthen the network between researchers in Denmark and globally.
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