HUPO initiative Proteome Biology of Stem Cells

by Albert Heck (Utrecht University, the Netherlands) and Jeroen Krijgsveld (EMBL, Germany)

The last years have seen many new and exciting breakthrough in the field of stem cells, among which the discovery of induced pluripotent stem cells arguably is the most important one. The formation of iPS cells has therefore moved to the center stage of research, including proteomics research. It has had also a significant effect on the projected international collaborations related to the HUPO initiative Proteome Biology of Stem Cells, where it has become an additional topic besides the more 'traditional' ones (e.g. pluripotency and differentiation). Progress on the crossroads of stem cells and proteomics were extensively discussed at:

1) The HUPO meeting in Amsterdam in 2008
2) The Korean Proteomics meeting on Jeju Island 2008
3) The International Society for Stem Cell Research (ISSCR) meeting in Barcelona in 2009
4) The Wellcome Trust/EBI Perspectives in Stem Cell Proteomics Conference 2009.

A dedicated Wellcome Trust/EBI meeting'Perspectives in Stem Cell Proteomics' was initiated by Rolf Apweiler and Albert Heck and co-organised by them and Richard Simpson and Michael Dunn at the Wellcome Trust Conference Centre in Cambridge, UK from Sunday 22nd - Monday 23rd March 2009. This conference focused on the current status of proteomics in stem cell research, from reviewing the state of the art in technology, to its potential in understanding of fundamental developmental biology as well as therapeutic implications. This meeting filled a niche, since stem cell biology and proteomics are both highly specialized scientific domains that rarely meet. The only way to bridge this gap and derive optimal benefit from what each field has to offer, is to bring together the specialists from both fields to discuss needs, possibilities, requirements and conditions that will have to be resolved before collaborative efforts can be successful. This conference aimed to facilitate this process. A 2 day training workshop took place prior to the conference. A more detailed description of this meeting has been published by E. de Wynter and R. Unwin *Proteomics*. 9 (2009) 3630-3634 and by J. Muñoz and A. J. R. Heck in Genome Med. 1 (2009) 45, which are attached.

As a follow up of this meeting several bilateral and international collaborations have been initiated, although not all of them necessarily under the umbrella of ISSCR or HUPO. The field is very lively and the last year has seen for instance several important phosphoproteomics applications in the field of (embryonic) stem cells.

Because of the density of meetings this year, especially the dedicated Wellcome Trust/EBI meeting and the ISSCR meeting in Barcelona, we have chosen not to convene another dedicated meeting at the HUPO meeting in Toronto.

DOI 10.1002/pmic.200900278 *Proteomics* 2009, *9*, 3630–3634

Wellcome Trust/EBI 2009 Meeting Report – Perspectives in Stem Cell Proteomics

REPORT

22–23 March 2009, Wellcome Trust Conference Centre, Hinxton, UK

Erika de Wynter[1] *and Richard Unwin*[2]

[1]Leeds Institute of Molecular Medicine, University of Leeds, St. James's University Hospital, Leeds, UK
[2]Stem Cell and Leukaemia Proteomics Laboratory, School of Cancer and Imaging Sciences, University of Manchester, Kinnaird House, Christie Hospital, Manchester, UK

This report summarises the recent "Perspectives in Stem Cell Proteomics" meeting that was held at the Wellcome Trust Conference Centre, Hinxton, UK in March 2009. The aim of the meeting was to explore the current status of proteomics in stem cell biology. Several themes encompassing technological and biological studies demonstrated the close relationship that must exist between the two communities in order to maximise our understanding of stem cell behaviour. Highlights included new methods for induction of pluripotent stem cells, new data sets regarding protein expression and phosphorylation dynamics in differentiating cells and the potential for future exploitation in a therapeutic setting.

Received: May 1, 2009
Revised: May 13, 2009
Accepted: May 13, 2009

Keywords:
Induced pluripotent stem cells / Phosphorylation / Stem cells

Introduction

This 2-day conference entitled "Perspectives in Stem Cell Proteomics" followed a 2-day proteomics training workshop held at the Wellcome Trust Conference Centre, Wellcome Trust Genome Campus, Hinxton, Cambridge. The conference aimed at bringing together those interested in the highly specialised fields of stem cell biology or proteomics and attracted more than 80 delegates (Fig. 1). The presentations from national and international leaders in their specialised scientific domains stimulated much discussion and should result in further collaborative efforts in these areas.

The conference was divided into four themes and this report aims to summarise the presentations under each theme. The opening remarks by Rolf Apweiler (The European Molecular Biology Laboratory (EMBL) European Bioinformatics Institute (EBI)) stressed the need for collaboration between the stem cell biology and proteomics communities as expertise from both sides is required to optimally extract biological information from complex proteomics data sets. Indeed, this point was highlighted throughout the course of the meeting. To further strengthen the links between these communities, Albert Heck described the "Proteome Biology of the Stem Cells Initiative", endorsed by the Human Proteome Organization (HUPO) and the International Society for Stem Cell Research (ISSCR).

On the cross-road of stem cells and proteomics

Alan Trounson (President, California Institute for Regenerative Medicine, CIRM) opened the meeting with a presentation of his vision for advancement of future stem

Correspondence: Dr. Erika A. de Wynter, Section of Molecular Gastroenterology, Leeds Institute of Molecular Medicine, Wellcome Trust Brenner Building, St. James's University Hospital, Leeds, LS9 7TF, UK
E-mail: e.a.dewynter@leeds.ac.uk
Fax: +44-113-3438702

Abbreviations: EBI, European Bioinformatics Institute; **EGF**, epidermal growth factor; **EMBL**, The European Molecular Biology Laboratory; **ESC**, embryonic stem cell; **FGF-2**, fibroblast growth factor 2; **HSC**, haematopoietic stem cell; **iPS**, induced pluripotent stem (cell); **LOPIT**, localisation of organelle proteins using isotope tagging; **PCA**, principle components analysis; **SILAC**, stable isotope labelling by amino acids in culture

Figure 1. Some of the participants of the Wellcome Trust/EBI 2009 meeting on "Perspectives in Stem Cell Proteomics", in Hinxton, Cambridge, UK.

cell work and the approach adopted by CIRM. The talk emphasised several important areas of research. The interaction between protein networks in induced pluripotent stem (iPS) cells generated by viral constructs needs to connect with proteomics. Recent studies indicate that pluripotent cells share protein–protein interaction networks that can influence pluripotency and self-renewal. Therefore, protein—protein interactions are important. The connection between stem cells, cancer and other diseases is being unravelled with the identification of proteins common to all three states. Many stem cell preparations show clinical promise and others are undergoing assessment in animal models. Of the $3 billion investment in stem cell work at CIRM, $1 billion has already been used to establish basic stem cell research and creation of partnerships with international groups in Canada, UK, Spain and Japan. A global approach was emphasised as the way forward in order to achieve a transition from basic stem cell work, through translational studies and finally clinical applications – a recurring theme throughout the conference.

Paul Simmons (Brown Foundation Institute of Molecular Medicine, Texas) discussed the challenges and opportunities for proteomic analysis of adult stem cells. Stem cells are extremely rare in adult tissues and cannot be propagated in culture, so obtaining sufficient cells for proteomics is a challenge. Proteomic analysis of populations enriched for murine haematopoietic stem cells (HSC) revealed that only 32% of protein changes correlated with mRNA. By searching for antigens common to both HSC and cells of their stromal environment, two potential new markers have been identified on human HSC, 3A9.F11 (podocalyxin) and BB9 (angiotensin converting enzyme). The importance of isolating cells prospectively for proteomics was emphasised as there are dramatic alterations in transcription after cells are cultured.

Christine Mummery (Leiden University Medical Centre, The Netherlands) shared some of her experiences in the culture of human embryonic stem cells (ESC) and their differentiation to cardiovascular lineages. Having examined the transcriptome and identified interesting genes, the next step was to use proteomics to (i) identify cell surface proteins useful for selection and purification of specific subtypes and (ii) identify active signalling pathways that control self-renewal and differentiation. The stable isotope labelling by amino acids in cell culture (SILAC) approach was used to identify changes in the proteome and phosphoproteome. These studies were described in more detail later in the meeting (see Heck and Krijgsveld). Data were also presented demonstrating that removal of feeder cells can markedly alter

the levels of specific proteins, such as vimentin. This is an important observation indicating that sample selection and preparation is a key step prior to protein analyses.

Anthony Whetton (University of Manchester) gave an elegant lecture introducing the techniques applicable to stem cell proteomics. As paradigms, he showed data from a study of *nanog* knockdown in embryonic stem cells, collaborating with Ihor Lemischka (Mount Sinai, New York) where as well as the proteome, changes in transcriptome and chromatin structure were measured, challenging the dogma that DNA makes RNA makes protein in a linear sequence of events. Their group demonstrated that a large proportion of protein changes during the initial stages of differentiation were in fact regulated post-translationally, including key *polycomb* genes and *nanog*-interacting proteins. Analysis of the chromatin proteome showed that certain histone subtypes may alter their binding to chromatin during differentiation. The final part of the presentation discussed methods for analysis of the phosphoproteome, using as a model the phosphorylation changes induced in primitive cells by the chemokine SDF-1, *via* the Rac G protein. Novel sites of phosphorylation were identified and validated, providing a powerful illustration of the kind of data that can be produced by proteomics analysis.

Protein markers of stem cells

Martin Pera (Eli & Edythe Broad Centre for Regenerative Medicine & Stem Cell Research, California) reminded the audience that unlike the murine system, we still do not know what promotes survival of human ESCs. Clonal growth from single cells is still fraught with problems and in culture the ESC markers vary depending on where the cells are in the colony. The microenvironment is also heterogeneous making it difficult to tease apart key components or cell–cell interactions. The ability of proteomics to perform targeted as well as global analyses means that it is potentially a key tool in the study of ESCs and their microenvironment. The essential needs of the ES community was summarised in three requirements for successful analysis: (i) targeted analysis of a modest number of proteins in depth, (ii) technologies adaptable to small numbers of cells and (iii) well-defined cell populations.

Albert Heck (University of Utrecht) posed two questions: do stem cells express specific proteins and what membrane proteins could be used as selectable markers of cells differentiating towards cardiomyocytes? The work presented indicated that the proteome of stem cells is also defined by culture conditions and different pathways are activated or deactivated during transition from mesoderm to cardiomyocytes. After careful choice of the best stem cell line for cardiomyocyte differentiation capacity, he outlined his approach to using proteomics to screen for cell surface markers useful for distinguishing stem cells from their differentiated counterparts. Some useful technical details and tips were provided on isolation, sample preparation, digestion of plasma membrane proteins and the importance of deglycosylation or delipidation. However, there may need to be some compromise (a balance needs to be attained) between purity of the preparations and the number of plasma membrane proteins that are detectable.

Andras Nagy (Samuel Lunenfield Research Institute, Toronto, Canada) presented an exciting talk of particular interest to stem cell biologists, on a novel non-viral way to generate iPS cells. The talk detailed use of the *piggyBac* transposon/transposase system to reprogramme murine and human somatic cells and generate stable iPS cell lines. This method may be the breakthrough for clinical applications as all four transcription factors are delivered in a single transposon and oncogenic transcription factors, such as c-Myc, can be removed subsequently from the cell line. Terminally differentiated iPS cells can be subjected to a second round of reprogramming to regenerate more iPS cells, in what was termed a massive "population-type" manner. The number of iPS cells generated in the second round should be sufficient for proteomics analysis leading to further exciting studies on stem cell maintenance and differentiation. Finally, a model "concept for conversion of a primary cell to an ESC" was presented and discussed.

Mahendra Rao (LIFE Technologies, USA) concluded the session by summarising some outstanding issues with proteomics: the number of detected proteins *versus* the proteome, running replicates, abundant proteins *versus* rare proteins, "one-hit wonders" and assigning peptides to the correct proteins, comparison of the proteome and transcriptome and SILAC issues. The need to use a focussed approach rather than whole genome was again emphasised.

Signalling in stem cells

Brian Hendrich (University of Cambridge) opened the second day of the conference describing work on members of the NuRD and Sin3a protein complexes. The complexes are important in ESC differentiation and the presentation outlined a variety of biochemical approaches to study how members of these co-repressor complexes interact. By using proteomics, key protein domains were identified in order to determine targets of the complex. In addition, novel interactions revealed *Sall4* as a component of NuRD complex in ESCs suggesting that the approach adopted has potential to reveal novel transcription factors controlling proliferation of ESCs.

Jeroen Krijgsveld (now at EMBL, Heidelberg) followed with an impressive description of work performed in Utrecht to analyse the phosphoproteome during exit from the pluripotent state induced by bone morphogenetic protein-2. Over 3600 unique phosphopeptides were identified using SILAC labelling. To understand this data, they used clustering tools to generate profiles of proteins whose phosphorylation patterns are similar, and used algorithms

to identify conserved kinase consensus motifs. Thus, CDK1/2 was identified as a key kinase regulator of this network. In addition, data were mapped onto known signalling cascades, revealing activation of the Jnk and Akt pathways. Although analysis of these data into a single defining model is ongoing, this talk clearly illustrated the immense challenges that exist beyond data generation.

Andre Choo (Bioprocessing Technology Institute, Singapore) continued the signalling theme by examining the role of fibroblast growth factor 2 (FGF-2) in human ESCs. Although the growth factor is essential in propagation of undifferentiated hESC in culture, the function of FGF-2 is unclear. FGF-2 stimulation activates all four FGF receptors in undifferentiated hESC but these receptors decrease at the protein level by day 12 of differentiation and this is not accompanied by similar changes in mRNA levels. Use of an FGF receptor inhibitor and inhibition of PI3 kinase reduced pluripotency markers. FGF-2 may have an impact on the Wnt signalling pathway where glycogen synthase kinase (GSK)-3 was phosphorylated, leading to translocation of beta-catenin to the nucleus and activation of Wnt signalling targets, including Nanog and Oct3/4. To further investigate the role of FGF-2 in ESC differentiation, discovery proteomics was used to identify targets of FGF-2 stimulation. Several proteins were identified, whose phosphorylation status alters rapidly upon FGF-2 stimulation, though the majority of these proteins were not components of the canonical FGF signalling pathway.

Blagoy Blagoev (University of Southern Denmark, Odense) closed the session by first discussing some of their excellent published work on epidermal growth factor (EGF) and platelet-derived growth factor treatment of human mesenchymal stem cells and a time course of EGF-stimulated HeLa cells. The latter study yielded quantitative information on some 6600 phosphorylation sites. This was followed by a discussion of work on three human ESC lines and cell differentiation, describing first of all the considerations for attaining complete SILAC labelling of such cells. A membrane proteome comparison to identify markers of ESCs was highlighted and several proteins identified, whose membrane expressions were at least threefold higher in undifferentiated cells. Interestingly, in this study the protein levels correlated well with mRNA levels. Finally, an impressive piece of work analysing a time course of human ESC differentiation was presented with an in-depth comparison of the proteome and phosphoproteome. Over 6500 proteins were identified and quantified as well as over 15 000 phosphorylation sites.

Stem cells and disease

Andre Terzic (Mayo Clinic, Rochester) opened the final session with a topical presentation on the practical use of stem cells for heart disease where the goal is to rebuild the muscle mass of the heart. One approach is to analyse proteins secreted from cardiogenic cells from the embryo and use the system as a paradigm to study the secretome of cells grown with or without specific differentiation media. This might enable hypotheses to be generated regarding key signalling pathways for differentiation of primitive cells into cardiac cells. A second approach is the induction of mesenchymal stem cells towards cardiogenesis using autologous cells from the patient, which has led to initiation of the "C-Cure Clinical Trial". A transcriptome approach to dissect the four stages of cardiomyocyte generation has already indicated that the chemokine receptor CXCR4 appears when pluripotential cells are losing their markers but before cardiac markers are evident. The presentation concluded with remarks on the potential use of iPS cells. Although patients with disease can generate disease-free iPS cells, the cardiogenic potential varies with each clone indicating careful and comprehensive analyses must be performed prior to therapeutic application of these cell lines. In summary, three platforms are envisaged for clinical use including guided cardiopoietic stem cells, well-characterised cardiac progenitors and iPS cells.

Richard Simpson (Ludwig Institute for Cancer Research, Melbourne) then turned to the use of mammary tissue as a model to isolate stem/myeloepithelial cells, luminal progenitors and differentiated cells according to cell surface markers. Details of membrane preparation, subfractionation, proteomic strategies and data analysis from each population were provided. One weakness of proteomics was outlined, which focussed on problems of trying to identify the protein that the peptides are derived from and to develop antibodies to identify that protein. The development of proteomic tools for in-depth analysis of the secretome and exosome was outlined and have already revealed changes in protein secretion, which are not accompanied by changes in total expression of protein or mRNA when cells undergo epithelial-mesenchymal transition. Stem cell specific proteins such as galectin-1 have also been identified. Peptidome analysis exclusively identified ectodomain shedding and regulated intramembrane proteolysis while the study of soluble secreted proteins from exosomes was proposed as a useful tool for the future. Urine and colon have specific exosomes suggesting a tissue specific signature of the exosomal proteins.

Kathryn Lilley (University of Cambridge) illustrated how plant-based studies could be used to address a question rarely considered by the proteomics community – that of protein subcellular localisation and more importantly changes in protein localisation upon biological perturbation. The presentation reviewed the localisation of organelle proteins using isotope tagging (LOPIT) system developed in the laboratory, where the distribution of a protein along a sucrose gradient is measured and compared with proteins of known localisation. PCA and profile similarity is then used to assign proteins of "unknown" location. The advantages of LOPIT include a steady-state position, simultaneous visualisation of organelles, the ability to identify genuine resi-

dents of organelles and provision of an unbiased snapshot of the cell with simultaneous assignment to organelles. This central theory can be expanded to "dynamic LOPIT" to see which proteins change their relative position on a PCA plot before and after perturbation to identify changes in subcellular localisation. The method can also be used for defining the localisation of multiprotein complexes, protein isoforms and post-translationally modified proteins.

Pritinder Kaur (Peter MacCallum Cancer Centre, Melbourne) closed the session with the final talk returning to stem cell biology and work on the epidermal stem cell population. Using HSC as a paradigm, stem and progenitor cells of the skin were isolated using a range of phenotypic markers. The potency of candidate human keratinocyte stem cells and their progeny were tested in a novel transplantation model that allowed tissue regeneration and enumeration of stem cell numbers. Some preliminary results using microarrays were presented relating to defining the molecular profile of these primitive skin cell populations. The genes identified in the different cell subsets correlated with the observed functional behaviour though the value of proteomics in this field is yet to be evaluated.

The current status of proteomics and the newer techniques being developed show that the field is well placed to exploit and study the expanding range of adult, embryonic and iPS stem cells. Improved processing techniques to cope with the wealth of data generated should increase our knowledge and understanding of self-renewal, differentiation, protein–protein interactions and signalling pathways in stem cells. However, future progress will depend on merging information on protein presence with protein functional effects and biological endpoints.

The authors have declared no conflict of interest.

Meeting report

Perspectives in stem cell proteomics

Javier Muñoz and Albert JR Heck

Address: Biomolecular Mass Spectrometry and Proteomics Group, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands.

Correspondence: Albert JR Heck. E-mail: a.j.r.heck@uu.nl

Published: 28 April 2009

Genome Medicine 2009, **1**:45 (doi:10.1186/gm45)

The electronic version of this article is the complete one and can be found online at http://genomemedicine.com/content/1/4/45

Abstract

A brief report on the Perspectives in Stem Cell Proteomics Conference, Hinxton, UK, 22-23 March, 2009.

Introduction

Stem cells receive broad attention in the scientific literature and in society in general. This has been inspired by their unique properties - the potential to self-renew and to differentiate into multiple lineages. Human embryonic stem cells (hESCs) have the ability to form all cells in the adult body once they receive the proper signals. The capability to control and direct differentiation *in vitro* would offer opportunities to develop treatments for diseases that cannot be treated today, especially in the area of regenerative medicine, where the aim is to replace damaged tissue. However, there are still many challenges before hESCs can be safely used for clinical applications. Moreover, societal and ethical issues need to be addressed before basic science in this area could be successfully translated into the clinic.

The field of proteomics has matured immensely in recent years, now allowing proteome biology investigation at reasonable throughput in all areas of cell biology. Proteomics researchers have started to chart the proteome of individual primary stem cells and stem cell lines and their differentiated derivatives, to define a subset of stem cell-specific proteins, or to identify differentiation-specific proteins that can be used as benchmarks for the intermediate or terminal steps of stem cell differentiation. Importantly, proteomics studies have shown that transcriptome analyses cannot fully explain developmental changes, most likely because they are unable to detect post-translational processes such as protein modifications and protein-protein interactions.

At present, stem cell biology and proteomics are both rather specialized scientific domains. Specialists from each field seldom meet. Thus, crucial opportunities may be missed for setting priorities and goals, and for maintaining consistent and optimized standards for research where these fields intersect, essential for an effective comparison of experimental data across different laboratories. In response, researchers from both fields have joined efforts in recent years to facilitate joined meetings and initiate collaborative research, resulting in a 'Proteome Biology of Stem Cells' initiative supported by both 'parent' organizations - the Human Proteome Organization (HUPO) and the International Society for Stem Cell Research (ISSCR). At the 'parent' meetings, specific parallel sessions are organized, such as the ISSCR meeting in Barcelona (2009) and the HUPO meeting in Amsterdam (2008).

Next to dedicated sessions/workshops at these meetings, it was felt that a smaller get-together of specialists would further enable researchers from both fields to bridge this gap and derive optimal benefit from what each field has to offer. On invitation by the European Bioinformatics Institute (EBI) and the Wellcome Trust, the organizers aimed to bring together specialists from both fields to discuss needs, possibilities, requirements and conditions that will have to be resolved before collaborative efforts can be successful. The organizers were Rolf Apweiler (EBI, Hinxton), Mike Dunn (UCD Conway Institute, Dublin, Ireland), Michael Dunn (The Wellcome Trust, London, UK), Albert Heck

(Utrecht University and Netherlands Proteomics Centre, The Netherlands) and Richard Simpson (Ludwig Institute, Melbourne, Australia). Throughout two days, approximately 100 participants from all over the globe gathered to present and discuss recent results. Here, some of the presented highlights are briefly summarized.

At the crossroads of stem cells and proteomics

Alan Trounson (California Institute for Regenerative Medicine (CIRM), San Francisco, USA) started the meeting by providing an overview of research performed at CIRM. As its director, Trounson gave warm support for proteomics in the stem cell field by noting that 'CIRM is committed to backing research in stem cells and regenerative medicine and recognizes the important role that proteomics will play in the success of this new medicine'. Paul J Simons (University of Texas, USA), former president of the ISSCR, focused on the unique properties of adult stem cells and the challenges faced by proteomics researchers when studying these cells. In contrast to, for instance, embryonic stem cells (ESCs), neural stem cells (NSCs) and mesenchymal stem cells (MSCs), stem cells from adult tissues, cannot be easily propagated *in vitro* and, therefore, the amount of cells that can be analyzed is limited. He showed that the identification of CD143 as a surface candidate marker for hematopoietic stem cells (HSCs) from human embryonic, fetal and adult hematopoietic tissues might, however, assist to enrich this stem cell population with a higher efficiency. Christine Mummery (Leiden University Medical Centre, The Netherlands) provided an overview of how proteomics has been intertwined in her stem cell research. Her laboratory focuses largely on the generation of human embryonic cardiomyocytes from hESCs, whereby proteomics was used to uncover early cardiomyocyte markers. She also pointed at the high potential of induced pluripotent cells (iPSs) derived from individual patients for regenerative purposes. The use of isobaric tags for relative protein quantification (iTRAQ) was applied to the study of ESC differentiation by Anthony D Whetton (University of Manchester, UK). In this multiplexed way, temporal changes in the proteome of differentiating ESCs could be monitored. Relative quantification of over 1,600 nuclear proteins, including many transcription factors (for example, Oct4, Sox2), was achieved. Comparison with mRNA-based assays, chromatin immunoprecipitation analysis of histone acetylation and RNA polymerase II binding during ESC development demonstrated only partial correlations. For example, the Polycomb gene family members were found to be regulated at the post-translational level, as were many members of the Nanog protein interactome. Whetton concluded that 'the analyses of stem cell protein networks require protein level analyses'. Mahendra Rao (LIFE Technologies and Buck Institute, CA, USA) further discussed the overall poor correlation between proteomic and genomic data, but he also felt that this still largely reflects technical difficulties in completely mapping the proteome space. In his view, protein expression analysis using phosphoprotein antibodies, protoarrays or examination of specific families of proteins had so far resulted in the greatest success, with high correlation with gene expression datasets.

Protein markers of stem cells

Martin F Pera (University of Southern California, USA) discussed the complexity of stem cells due to their microenvironment. hESC cultures are heterogeneous, consisting of a spectrum of cells at various stages in a hierarchy of developmental potential. These cells live in discrete compartments within the culture, they communicate with one another, and they can be identified by their expression of extracellular matrix molecules, cell surface markers, and growth factors. Pera concluded that the hESC microenvironment is a fertile area for proteomics investigation, if the technology can address the issues of limited sample size, low abundant proteins, and complex post-translational modifications. Albert Heck focused on the identification of cardiomyocyte-specific cell surface markers by proteomics, which may potentially be used for sorting purposes during early stages of their *in vitro* propagation from hESCs. Dedicated protocols to enrich membrane proteins, applicable to minute amounts of stem cells, were discussed. Putative markers could be distilled from a comparative analysis of hESC-derived cardiomyocytes and cardiomyocytes derived from fetal heart tissue, using stable isotopes of amino acids in cell culture (SILAC) to label the hESCs. One of the most recent breakthroughs in the field of stem cell research has been the finding that cells may be reprogrammed to a pluripotent state (iPS) by bringing in a few stem cell-specific transcription factors; c-Myc, Klf4, Oct4 and Sox2. Initially, low-efficient, plasmid-based delivery methods and more efficient viral vectors were used, whereby the latter pose a risk of uncontrollable insertional mutagenesis with related tumor genesis risks. Andras Nagy (Mount Sinai Hospital, Canada) showed a method to overcome these issues, making use of the *piggyBack* transposon/transposase system to deliver the reprogramming factors. Interestingly, this method allows the removal of the transposon insertions from established iPS cell lines, providing a unique tool for high-throughput technologies such as proteomics to investigate the molecular basis of the cellular reprogramming process. Nagy could show that the cells could be reprogrammed in a synchronous way.

Signaling in stem cells

The mechanisms controlling the differentiation process of ESCs are poorly understood. Using SILAC-based quantitative mass spectrometry (MS), Jeroen Krijgsveld (University of Utrecht, now at the European Molecular Biology Laboratory (EMBL), Heidelberg, Germany) and Blagoy Blagoev (University of Southern Denmark, Denmark)

analyzed the (phospho)proteome of human embryonic stem cells following induced differentiation. The results presented, covering several thousand phosphoproteins simultaneously, revealed several important changes in the pluripotent core regulatory networks. Krijgsveld showed for Sox2 a delicate interplay between SUMOylation and phosphorylation, providing new insights into how hESCs potentially exit the pluripotent state. From the work of both Krijgsveld and Blagoev, the intricate cross-talk between signaling pathways in stem cell differentiation became clearly apparent.

Stem cells and disease

Because of their potency, stem cells might be used as a source of tissue replacement in regenerative medicine. However, many issues are nowadays faced by clinicians who attempt to use pluripotent cells in practice. Andre Terzic (The Mayo Clinic, MN, USA) discussed many of them and showed some results of his research where proteomics and other high-throughput technologies took a central role. Different approaches were discussed, ranging from the use of sorted cardiogenic progenitors from ESCs or autologous reprogrammed iPSs to repair cardiac function in patients with myocardial infarction. The stem cell niche, known as the microenvironment in which stem cells are found and that acts in regulating cell fate decisions, still remains largely unexplored. Richard J Simpson (Ludwig Institute for Cancer Research, Melbourne, Australia) described proteomics approaches that dealt with the secretome (secreted soluble proteins), exosome (secreted membrane vesicles) and peptidome (natural occurring peptides) during the epithelial-mesenchymal transition.

Concluding remarks

The meeting ended with a retreat in which researchers, funding agencies (EU, Wellcome Trust, Genome Canada) and publishers discussed several issues and opportunities, in the perspective of cross-disciplinary research in this area. Small in size, the meeting was a great success at initiating discussions that will ultimately lead to more collaborative efforts at the crossroads of stem cell and proteomics research.

Abbreviations

CIRM, California Institute for Regenerative Medicine; EBI, European Bioinformatics Institute; EMBL, European Molecular Biology Laboratory; ESC, embryonic stem cell; hESC, human embryonic stem cell; HSC, hematopoietic stem cell; HUPO, Human Proteome Organization; iPS, induced pluripotent stem cell; ISSCR, International Society for Stem Cell Research; iTRAQ, isobaric tags for relative protein quantification; MS, mass spectrometry; MSC, mesenchymal stem cell; NSC, neural stem cell; SILAC, stable isotopes of amino acids in cell culture.

Competing interests

The authors declare that they have no competing interests.