

Meeting report

Perspectives in stem cell proteomics

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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 micro-environment. 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 micro-environment 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.