Preliminary Program, as of Feb 19, 2009

2nd Bioinformatics Satellite Workshop,
right after the 5th German meeting on Stem cells,

on Thursday March 26, 2009, Helmholtz Institute for Biomedical Engineering, Pauwelsstrasse 20, 52074 Aachen

9:25h Welcome

9:30h F.-J. Müller: Surfing the PluriNet: Pluripotency V2.0

10:00h T. Schroeder: Tracking stem cell behavior and molecular expression at the single cell level: New tools for old questions

10:30h Discussion time / Coffee

11:15h W. Wagner: Aging and Replicative Senescence Have Related Effects on Human Stem and Progenitor Cells

11:45h Q. Lin: A Network-based Approach for Analyzing Pathway Interactions

12:15h Discussion time / Lunch break

14:00h M. J. Araúzo-Bravo: Perspectives on the Application of Metabolic Flux Analysis to Embryonic Stem Cells

14:30h G. Fuellen: Which analyses are in demand for [a 2-day Practical Course in] 'Stem Cell Bioinformatics'?

15:00h Last-Minute / Ad-Hoc / Lightning Talks: L. Scheubert, S. Struckmann, …

15:30h Discussion time / Coffee

16:00h Informal Get-together: A Master Plan towards an in-silico representation of pluripotency…

17:00h Closing (We plan to have dinner around 18:00h.)

Surfing the PluriNet: Pluripotency V2.0

Franz-Josef Müller, Igor Ulitsky, Louise C. Laurent, Insa Lenz, Andreas Jeske, Imke Petersen, Josef Aldenhoff, Jeanne F. Loring

Center for Integrative Psychiatry ZIP gGmbH, Kiel, Germany.

In recent years, the concepts of stem cell self-renewal and developmental potential have changed considerably. Increasingly powerful methodologies that can survey cell processes on a genome-wide scale are overtaking single gene approaches for explaining complex phenomena such as pluripotency. We recently reported the creation and analysis of a database of global gene expression profiles (the Stem Cell Matrix) that enables the classification of cultured human stem cells in the context of a wide variety of pluripotent, multipotent and differentiated cell types. We used further bioinformatic analysis to uncover a protein–protein network (PluriNet) consisting of 299 interacting proteins that is shared by the pluripotent cells (embryonic stem cells, embryonal carcinomas and induced pluripotent cells). Analysis of published data showed that this protein interaction network appears to be a common characteristic of many types of pluripotent cells, including mouse embryonic stem and induced pluripotent cells and human oocytes.

A new challenge has arisen because of our increasing ability to generate and computationally analyze large biological data sets: how should the large-scale molecular hypotheses that emerge from these data, such as the PluriNet, be experimentally tested in the laboratory? We will discuss what biological questions have emerged from the Stem Cell Matrix and PluriNet and how these are being experimentally addressed.

Tracking stem cell behavior and molecular expression at the single cell level: New tools for old questions

Timm Schroeder

Institute of Stem Cell Research, Helmholtz Zentrum Muenchen - German Research Centre for Environmental Health (GmbH), Munich / Neuherberg, Germany.

Despite intensive research, many long-standing questions in stem cell research remain unsolved. One major reason is the fact that cell fates are usually followed by analyzing the fate of populations of cells - rather than individual cells - at very few time points of an experiment, and without knowing their individual identities. Real-time tracking of individual cells in culture, tissues or whole organisms would be an extremely powerful approach to fully understand the developmental complexity of stem cell systems. However, many required tools are still under development and their application remains difficult. We have developed a computer aided culture and imaging system to follow the fate of individual cells over long periods of time. New software was programmed, helping to record and display the divisional history, position, properties etc. of all individual cells in a culture over many generations. I will discuss how we used this system to analyze self renewal and differentiation of stem cells in a quantitative way. In addition, I will demonstrate how this technology can be applied to analyzing expression levels of proteins of interest in living cells over time.

Aging and Replicative Senescence Have Related Effects on Human Stem and Progenitor Cells

Wolfgang Wagner, Simone Bork, Patrick Horn, Thomas Walenda, Anke Diehlmann, Vladimir Benes, Jonathon Blake, Franz-Xaver Huber, Volker Eckstein, Anthony D. Ho

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Institute for Biomedical Engineering, Aachen University Medical School, Germany.

The regenerative potential diminishes with age and this has been ascribed to functional impairments of adult stem cells. Cells in culture undergo senescence after a certain number of cell divisions whereby the cells enlarge and finally stop proliferation. This observation of replicative senescence has been extrapolated to somatic stem cells *in vivo* and might reflect the aging process of the whole organism.

We have addressed the impact of replicative senescence on mesenchymal stromal cells (MSC) from human bone marrow. Within 43 to 77 days of cultivation (7 to 12 passages), MSC demonstrated morphological abnormalities, enlargement, attenuated expression of specific surface markers, and ultimately proliferation arrest. Adipogenic differentiation potential decreased whereas the propensity for osteogenic differentiation increased. Gene expression profiles were analyzed by Affymetrix GeneChip technology and this revealed a consistent pattern of alterations in the signature of MSC at different passages. These changes are not restricted to later passages, but are continuously acquired with increasing passages. In continuation of this work, we have analyzed effects of aging on gene expression profiles of MSC or of human hematopoietic progenitor cells (HPC). MSC were isolated from bone marrow of 12 donors that were between 21 and 92 years old. To identify differential gene expression that correlated with increasing age we have performed Pavlidis template matching (PTM). 67 genes were significantly age-induced and 60 were age-repressed ($P<0.01$). $CD34^+$ HPC were isolated from cord blood of 4 donors and from mobilized peripheral blood of 15 healthy donors between 27 and 73 years. PTM analysis revealed that 432 genes were age-induced and 495 were age-repressed. The overlap of age-associated differential gene expression in HPC and MSC was moderate. However, it was striking that several age-related gene expression changes in both HPC and MSC were also differentially expressed upon replicative senescence of MSC *in vitro*. Especially genes involved in genomic integrity and regulation of transcription were age-repressed.

These studies have demonstrated that aging causes gene expression changes in human MSC and HPC that vary between the two different cell types. Changes upon aging of MSC and HPC are related to those of replicative senescence of MSC *in vitro* and this supports the notion that our stem and progenitor cells undergo replicative senescence also *in vivo*.

A Network-based Approach for Analyzing Pathway Interactions

Lin Qiong

Institute for Biomedical Engineering, Department of Cell Biology, Rheinisch-Westfälische Technische Hochschule Aachen University, Aachen, Germany.

MOTIVATION: Understanding the structure and dynamics of the complex intra-cellular web of interactions has become a central focus of biological research. A network-based approach offers the possibility to understand the cell as a system. A promising approach towards understanding network-based biology is the generation of Protein-Protein Interaction (PPI) networks, which could illustrate protein functions in a defined biological context. Cell signaling pathways can interact (crosstalk) with each other to integrate and regulate information flow. This phenomenon has long been considered as an important determinant of cellular response in health and disease. Since PPIs are fundamental to the interconnections between two pathways, interactions among pathways can be detected by systematically integrating pathway data and human PPI data. Therefore, network-based methods can be applied to model pathway interactions. The goal of the study was to reconstruct a global pathway interaction network to understand interconnections among human cell signaling pathways. RESULTS: Properties of the pathway interaction network (i.e. scale-free) are characterized, and significant pathway clusters are identified and visualized.

The intensively interconnected pathways are detected as well. Furthermore, by mapping microarray data onto the network, corresponding modules are built for further investigation. Such a network is a computational framework for modeling cells as an integrated system, and can be applied to model pathway interactions in stem cells.

Perspectives on the Application of Metabolic Flux Analysis to Embryonic Stem Cells

Marcos J. Araúzo-Bravo

Max Planck Institute for Molecular Biomedicine, Department Cell and Developmental Biology, Muenster, Germany.

Metabolic engineering has promoted research on systems-oriented methodologies such as Metabolic Flux Analysis (MFA). MFA provides a holistic perspective on metabolism that allows a quantitative understanding of biochemical reaction networks. The simplest conventional method of MFA relies only on the measurement of specific extracellular rates. It is based on stoichiometric equations with mass balances. However, this approach has the limitation that the fluxes through parallel and cyclic pathways, as well as bidirectional pathways, cannot be estimated. An alternative approach to cope with these limitations is based on tracer labeling experiments.

Reliability and resolution of metabolic fluxes can be enhanced using additional information from labeling experiments. The labeling experiments provide NMR (Nuclear Magnetic Resonance) or MS (Mass Spectrometry) measurements. Two strategies are commonly used to deal with labeling information. The simpler one is the calculation of flux ratios at important branch points of metabolic networks. The more complex but powerful approach can estimate all the fluxes from NMR and MS experiments by applying an inversion method that uses the tentative sets of independent fluxes to simulate the labeling state of the metabolites and compare this with the measured data.

We have developed an integrated software package with a graphic user interface, which covers all the stages of the MFA method. Namely, the software covers the phases from modeling of biochemical networks and the input of measurements, through the preprocessing phase including optional correction of non-steady state and natural labeling, and the processing including genetic algorithms for overcoming the local minima problem of the non-linear optimization associated with the metabolic fluxes estimation, to the post-processing which includes statistical analysis for estimating the confidence intervals of the fluxes. The software has also the ability of automated report generation in LaTeX and HTML formats.

The package has been validated in the study of the metabolism of prokaryote systems. A remaining challenge is the extension of MFA to the study of the metabolism of eukaryote systems. Here we will analyze the difficulties and possibilities of applying the MFA techniques to the study of Embryonic Stems Cells (ESC), as well as the opportunities that could be gained from such application.

Which analyses are in demand for [a 2-day Practical Course in] ‘Stem Cell Bioinformatics’?

Georg Fuellen

Institute for Medical Informatics and Biometrics, Department of Medicine, University of Rostock, Germany.

Reviewing the literature, I will try to give an overview of ‘stem cell bioinformatics,’ and I will try to come up with a definition. I will summarize the various types of data, and the most relevant biological questions being asked. I will present some ‘role models’, where analyses of the data yielded important insight. I will discuss the techniques used for data analysis, and try to highlight the most promising combinations of data, questions and analysis methods. My review shall yield conclusions on where the future of bioinformatics is, in the area of stem cell data analysis. These conclusions shall also influence the contents of a practical course in ‘stem cell bioinformatics’ that is planned for the fall (in Rostock), primarily as a service for the DFG priority program on ‘Pluripotency and Cellular Reprogramming’.