LATE ABSTRACTS

3rd Satellite Workshop on Bioinformatics in Stem Cell Research
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MAPPING THE HUMAN EMBRYOME

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Human ES or iPS-derived cellular therapeutics will likely require unprecedented standards of quality control, including stringent characterization and documentation of identity and purity. In an effort to generate highly purified, diverse, and scalable embryonic progenitor (EP) cell types for potential use in human cell therapy, we undertook the large-scale combinatorial cloning of EP cell lines from the normal human ES cell lines WA09/H9 and MA03/ACT03. We reasoned that the clonal isolation of primitive EP cell lines would markedly reduce the fate space of the lines thereby simplifying the purification of clinical-grade product and identification by transcriptome profiling. We isolated approximately 1,100 diverse candidate human EP cell lines and characterized 242 of the better growing lines by Illumina Human-6 v1 & v1 and Affymetrix U133 Plus 2.0 microarrays. Non-negative matrix factorization profiling detected 140 distinct cell types within the 242 cell lines. These lines display a wide array of markers of primitive endodermal, mesodermal, ectodermal, and neural crest types with diverse site-specific homeobox gene expression. A fate space screening protocol utilizing approximately 100 of these EP lines are yielding novel differentiation protocols of potential clinical import. Three EP lines show a robust induction of chondrogenic gene expression during differentiation including the expression of COL2A1, LECT1, and EPYC. Immunocytochemical and histological analysis confirmed chondrogenesis and the compatibility of the cells with biodegradable matrices. The three chondrogenic lines showed diverse site-specific homeobox genes with one being LHX8+ BARX1+, one HOXA2, B2+, and one lacking HOX gene expression. Unlike bone marrow-derived mesenchymal stem cells, none of the chondrogenic lines expressed CD74 or the hypertrophic chondrocyte marker IHH. The complexity observed in hES-derived clonal EP cell lines underscores the need for a detailed map of the cell surface antigens, other diverse molecular markers, and ontological tree of the cells of human development. In addition, the diversity, scalability, and relative stability of clonal hES-derived embryonic progenitor cell types offers a novel avenue for the study of gene regulatory networks and signaling pathways involved in early embryogenesis. Such data if linked to an online database would benefit stem cell research by facilitating the production of purified cell types of interest and eventually manufacturing protocols increasing the purity of clinical-grade cell-based therapies.

Quality control of cell reprogramming using DNA microarray

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The reprogramming of somatic cells to a pluripotent state established itself as a common practice in research labs. The potential use of such cells includes clinical application in regenerative medicine and drug screening in pharmacological research. The "gold standard" to assess pluripotency is the chimera formation; but such essay is very demanding and only applies to non-human cells. The National Institute of Health (NIH) and the International Society of Stem Cell Research (ISSCR) guidelines criteria for human pluripotency include the capability for teratoma formation, the global gene expression similar to embryonic stem cells (assessed by gene-expression microarrays), and the transcription factors and other genes activities associated with pluripotency. Then it is a common practice to use gene expression microarray assays as one of the tools to estimate the pluripotent capability of the induced pluripotent stem (iPS) cells. A common practice in the literature referring to global gene expression results is the use of terms such as "identical gene expression as embryonic stem cells (ESC)" or "similar gene expression to ESC" to qualify one's microarray results without a clear quantification of what they consider as "similar" or "identical". To standardize such terms in the comparison between iPS and other types of pluripotent cells with control ESCs, we propose to quantify such terminology using several indices that measure the degree of similarity between the new iPS populations and the ESC reference populations. Using such indices we predict whether pluripotent-induced populations really reach multipotent or pluripotent state, thus avoiding tedious and time-consuming biological experiments. The development of this approach became possible due to the growing corpus of microarray data of induced multipotent and pluripotent cells in both mouse and human models deposited in public databases. To envisage to which degree similarities and dissimilarities are significant, we compare and evaluate the reprogramming level achieved by several research groups from different starting material and using different microarray platforms by performing a microarray meta-analysis based on several quantitative indices. The main one, termed "potency index", measures how pluripotent is a cell population. A higher potency of the population corresponds to a higher potency index. This methodology has helped us discover cell types that although initially well-claimed to be pluripotent, do not show a global gene expression pluripotent profile.

Understanding pluripotency mechanisms and predicting small-molecule effects by integrative bioinformatics

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We are developing the *PluriNetWork*, a large network of interaction and regulation links between genes/proteins involved in pluripotency, and the *Expressence* software to highlight links in networks such as the *PluriNetWork,* attempting to infer mechanisms from differential experimental data such as microarray time series. Mechanisms important for pluripotency may be discovered by this kind of data integration, and small molecules close to genes involved in these mechanisms may be predicted to enhance the induction of pluripotency. Using networks and associated time series, etc, data describing various differentiation processes, we also wish to suggest small-molecule treatment schemes for transdifferentiation. We will finally describe how a community effort may be launched in network development and curation, based on merging wiki and social networking software. Maybe the workshop convenes a critical mass of interested people so that we can start right away…!