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Cytomics, the human cytome project and systems biology: top-down resolution of the molecular biocomplexity of organisms by single cell analysis

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Abstract. A large amount of structural and functional information is obtained by molecular cell phenotype analysis of tissues, organs and organisms at the single cell level by image or flow cytometry in combination with bioinformatic knowledge extraction (cytomics) concerning nuclei acids, proteins and metabolites (cellular genomics, proteomics and metabolomics) as well as cell function parameters like intracellular pH, transmembrane potentials or ion gradients.

In addition, differential molecular cell phenotypes between diseased and healthy cells provide molecular data patterns for (i) predictive medicine by cytomics or for (ii) drug discovery purposes using reverse engineering of the data patterns by biomedical cell systems biology. Molecular pathways can be explored in this way including the detection of suitable target molecules, without detailed *a priori* knowledge of specific disease mechanisms. This is useful during the analysis of complex diseases such as infections, allergies, rheumatoid diseases, diabetes or malignancies.

The top-down approach reaching from single cell heterogeneity in cell systems and tissues down to the molecular level seems suitable for a human cytome project to systematically explore the molecular biocomplexity of human organisms. The analysis of already existing data from scientific studies or routine diagnostic procedures will be of immediate value in clinical medicine, for example as personalized therapy by cytomics.

INTRODUCTION

The molecular biocomplexity of organisms may be investigated bottom-up from genes to biomolecules, and organelles to cells, tissues and organs (Collins *et al*. 2003), for example by systems biology. Systems biology concerns the analysis of the relationships amongst the elements in a system in response to genetic or environmental perturbations, with the goal of understanding the system or the emergent properties of the system, to mathematically model the system and to predict its behaviour towards external stimuli (Hood 2003; Weston & Hood 2004; Kelley & Ideker 2005). Essential characteristics of a system have to be known prior to specifically addressing disease processes in this typically bottom-up orientated approach.

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Given the high biocomplexity of mammals, resulting from the multiple heterogeneities at the genomic, cellular and tissue level, including the many exposures of biostructures to variable influences during life, it remains presently doubtful whether typical human disease processes like infections, allergies, diabetes or malignancies can be efficiently explored in this way within reasonable time intervals to provide practical benefits for individual patients. Systems biology data are typically obtained by high throughput technologies from cell extracts that is after destruction of cell integrity and cell environment in the original tissue. Under these conditions it is not always certain that afterwards observed molecular properties adequately reflect their *in situ* comportment.

THE TOP-DOWN SINGLE CELL ANALYSIS ORIENTATED CONCEPT

Single cell analysis by image or flow cytometric methods has equally reached high throughput capacity in recent years, permitting the assessment and quantification of the molecular morphology of single cells in suspension as well as in cell culture, cell smears or tissue sections. Typical investigations use hypothesis-driven parameter selection in combination with hypothesis-free exhaustive knowledge extraction (cytomics). While a given hypothesis can be proven or disproved by a given experiment, the evaluation of all collected cell data in a hypothesis-free fashion (discovery science) enables the exploration of unknown multiparametric data spaces. The secondary results obtained in this way are subsequently available for new hypothesis development and further experimentation. This approach respects the reality that cells and not genes or biomolecules represent the elementary functional units of organisms and that diseases are caused by molecular alterations in cells or cellular systems (cytomes) as consequence of genotype and exposure to external or internal influences.

A multitude of single cell features can be simultaneously captured by high throughput single cell microscopy (Bocsi *et al*. 2004; Ecker *et al*. 2004a, b; Gerstner *et al*. 2004; Kantor *et al*. 2004; Perlman *et al*. 2004; Schubert 2004; Mittag *et al*. 2005) and reconstituted to single cell molecular 3D tissue architectures (tissomics) (Ecker & Tarnok 2005; Kriete & Boyce 2005; Schubert 1990; Schubert 2004). High throughput flow cytometry (Edwards *et al*. 2004) or flow and image hybrid systems (George *et al*. 2004) as well as chip-based flow systems (Palkova *et al*. 2004; Weston & Hood 2004; Wu *et al*. 2004), cellular genomics (Taylor *et al*. 2004), cellular proteomics by immunophenotyping (Maynadié *et al*. 2002; Casanovas *et al*. 2003; Valet *et al*. 2003a; Habib & Finn 2005) and chemical cytometry (Dovichi & Hu 2003; Wu *et al*. 2004; Arkhipov *et al*. 2005) as well as cellular metabolomics (Dovichi & Hu 2003) constitute further facets of recent extensions in molecular cytomics.

When concentrating, for example, on disease processes, the comparison between cells of diseased and non-diseased persons provides nature-induced differential molecular cell phenotypes for affected or disease-associated cells such as inflammatory leukocytes. Differential molecular cell phenotypes in form of differential data patterns directly reflect the actual disease process at the cellular level not only for diagnostic but also for individually predictive purposes such as therapy-dependent future disease course predictions in individual patients (Gerstner *et al*. 2003; Valet & Tarnok 2003b). Molecular reverse engineering of such data patterns by biomedical cell systems biology should provide information on disease inducing molecular pathways, thus favouring the detection of new target molecules for drug discovery.

The relative inefficiency of recent drug discovery efforts, following a paradigm shift from the traditional *physiology* orientation towards the *molecular target* orientated approach (Sams-Dodd

2005), generated significant failures such as the low-density lipoprotein cholesterol lowering anti-atherosclerosis drug cerivastatin (Lipobay) (Psaty *et al*. 2004) or the anti-inflammatory cyclo-oxygenase 2 (COX2) inhibitors (Melnikova 2005). These experiences may induce a shift of efforts (Schneider 2004) towards the search for drugs effective on *distributed targets* as for example, salicylic acid acting on various molecular targets simultaneously. Such a shift will be facilitated by cytometric techniques favouring the analysis of reactive cellular protein networks as multitarget structures *in situ* (Grygierzec *et al.* 2004; Schubert 2004).

Molecular cell phenotypes provide, furthermore, the potential to systematically uncover organismal biocomplexity at the level of its basic function unit, the cell, thus enabling the establishment of a standardized periodic system of cells, tissue components and disease states at the biomolecule level in a human cytome project (Valet *et al.* 2004; Valet 2005). A detailed periodic system of cells would be of immanent practical value in stem cell biology for embryonic (Rippon & Bishop 2004) as well as for adult stem cell research (Camargo *et al.* 2004).

CONCLUSION

Considering the rapid development of highly sensitive, single-cell high-throughput methodologies during recent years, it seems important to increasingly orientate towards single cell and single patient research strategies to dissect the molecular biocomplexity of organisms into manageable portions. Cytometric techniques assure cellular integrity and high molecular resolution at the level of cells as the elementary function units of organisms. Because the top-down cytomics strategy of determining differential molecular cell phenotypes in diseased or disease-associated cells or cell systems does not depend on detailed *a priori* knowledge of disease mechanisms, the exploration of organismal biocomplexity is significantly simplified.

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