

IDENTIFICATION OF FUNCTIONAL CELL STATES

FIELD OF INVENTION

Embodiments relate to fields of cell assays, physiology and drug development. Embodiments additionally relate to cytometry and to semi-automated and automated analysis of multi-parametric data, such as, cytometry data.

GOVERNMENT FUNDING

No government funds were used in making the invention herein disclosed and claimed.

RELATED APPLICATIONS

This application claims priority of and incorporates herein in its entirety US Provisional Application number 61 /927,247.

SUMMARY

In one aspect, there is provided a method for characterizing the effect of an agent on cells comprising: a) contacting samples of a population of cells with a series of different concentrations of an agent; b) measuring each of two or more phenotypic parameters in individual cells of the population for each sample and for each exposure concentration; c) obtaining a distribution of measured values for the cells for each parameter and each exposure; d) obtaining a distribution of measured values for the cells not exposed to the compound; e) calculating dissimilarities between the distribution obtained in step d) and each of the distributions obtained in step c); f) combining the calculated dissimilarities to form a multiparametric response tensor; wherein the multiparametric response tensor is a fingerprint characteristic of the effect of the agent on the cells which can be directly compared to other fingerprints generated the same way.

DESCRIPTION

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Phenotypic compound screening is an emerging technology for rapid assessment of pharmaceutical compounds, in recent years, a number of techniques have been developed to characterize phenotypic responses of cells to perturbants such as small molecules or biologics. The vast majority of reported work has used traditional bulk biochemical assays, or single-cell techniques based on high-content screening (automated microscopy). For instance, see Abraham et al. ("High content screening applied to large-scale cell biology." *Trends Biochemol.* 22, 15-22, 2004) and Giuliano et al. ("Advances in High Content Screening for Drug Discovery." *ASSAY Drug Dev. Technol.* 1. 565-577, 2003), which are incorporated by reference herein in parts pertinent thereto.

More recently, novel statistical methods have been implemented in the analysis of complex screening datasets. These methods can provide a means to determine correlations between datasets. Simple pathway-driven models for screening are described in Cong et al ("Method for using division arrested cells in screening assays," EP 1581645), which is incorporated by reference herein in parts pertinent thereto, Cong proposes studying signal transduction in growth-arrested cells and using such systems to screen for agents that modulate the activity of cell surface receptors such as the β 2 adrenergic receptors (p2AR), a type of G-protein coupled receptor. Cong demonstrates that even in

What is claimed is:

1. A method for characterizing the effect of an agent on cells comprising:
 - a) contacting samples of a population of cells with a series of different concentrations of an agent;
 - b) measuring each of two or more phenotypic parameters in individual cells of the population for each sample and for each exposure concentration;
 - c) obtaining a distribution of measured values for the cells for each parameter and each exposure;
 - d) obtaining a distribution of measured values for the cells not exposed to the compound;
 - e) calculating dissimilarities between the distribution obtained in step d) and each of the distributions obtained in step c);
 - f) combining the calculated dissimilarities to form a multiparametric response tensor;wherein the multiparametric response tensor is a fingerprint characteristic of the effect of the agent on the cells which can be directly compared to other fingerprints generated the same way.
2. A method according to claim 1, wherein the method is used to generate and store fingerprints.
3. A method according to claim 1, wherein the method is used to make comparisons of fingerprints.
4. A method according to claim 3, wherein the comparisons are used to predict *in vivo* effects.
5. A method according to any one of the preceding claims, wherein the phenotypic parameters are measured in the individual cells by cell cytometry.
6. A method according to any one of the preceding claims, wherein the phenotypic parameters include any two or more of cell viability, cell cycle stage, mitochondrial membrane integrity, mitochondrial toxicity, glutathione concentration, reactive oxygen species, reducing species, cytoplasmic membrane permeability, DNA damage, a stress response marker, an inflammatory response marker, an apoptosis marker and a lipid peroxidase.
7. A method according to any one of the preceding claims, wherein the phenotypic parameters include any one or more of NF κ B, caspase, ERK, SAPK, P13K, AKT, a Bcl-1 family protein, p38, ATM GSk3B and ribosomal S6 kinase.