44. Bioinformatics tools to encode and integrate microscopy time-lapse sequences for drug discovery: Lineage analysis the basis for novel cell-based assays

Imtiaz A. Khan1, Lee Campbell2, Paul J Smith2 and Rachel J Errington3

Imtiaz A. Khan; wpciak@cf.ac.uk

1 Biostatistics and Bioinformatics Unit, School of Medicine, Cardiff University, Heath Park, Cardiff, UK;
2 Department of Pathology, School of Medicine, Cardiff University, Heath Park, Cardiff, UK;
3 Department of Medical Biochemistry and Immunology, School of Medicine, Cardiff University, Heath Park, Cardiff, UK

Cell lineage is defined as the dynamics of cell division in the development and maintenance of a population of cells, such as a tumour. The effectiveness of an anticancer drug depends on its ability to modulate tumour cell proliferation. An anticancer drug usually exerts its effect by interacting and manipulating different phases of cell cycle. Exploring and exploiting the enormous potential for pharmacological modulation of the mammalian cell cycle are key goals for basic research and drug discovery. We describe here a novel advance for cell-based screening of drug action -the temporal monitoring of cell cycle progression, enabling the tracking of single cell lineage progression and checkpoint transitions in a non-invasive manner even within heterogenous populations. Many drugs differentially target across the cell cycle, therefore this heterogeneity in cellular responses presents a clear route for cell cycle-mediated drug resistance.
Lineage analysis provides an elegant, non-invasive assay for understanding the complex interplay for tumour survival at the single cell level.

Time-lapse microscopy concept is the repeated collection of a single field of view from a microscope at discrete time intervals. The information contained in the field of view is determined by the method of contrast and includes phase and fluorescence. Transmission phase offers a probeless and relatively non-perturbing contrast mode which provides information on cell morphology (essentially cell shape and cell position), the changes in these two basic features facilitate assays describing dynamic cell behaviour interacting on a two-dimensional substrate. The duration of the time interval defines the temporal resolution, which in turn characterises the type of event detected. At the core of the assays is the implementation of time-lapse microscopy to link the initial cell cycle position during acute exposures to anti-cancer agents with the antiproliferative consequences for individual cells. These type of data are rich in information however a significant challenge to the cell biologist is developing tools which facilitates data reduction and data encoding providing a means for hypothesis driven data mining. Here we introduce novel bioinformatics tools for the mapping and analysis of critical cell cycle events in cell lineages.

Extracting the event lineages from the image data is not trivial and in some cases is difficult to automate, therefore we have developed bioinformatics tools which allows for both automated and manual input of event parameters. We have developed a user friendly graphical interface that aids the experimentalist to orientate themselves in the data since the growth of the lineage tree is in response to data input. Cell lineage data encrypting from the raw images, visualization and analysis in relation to drug treatment is the primary objective of this software. Written in Perl, the software has two core components, data generation and data analysis, both consist of a comprehensive graphical visualization

of cell lineage. In addition to the concurrent lineage tree growth, the input phase tags and classifies all possible event outcomes – such as the generation of two, three and four daughter cells as a result of mitotic commitment, or indeed the polyploidy
consequence as a result of unsuccessful chromosomal segregation after mitosis. The other
primary event recorded in this type of analyses is cell death (apoptosis or necrosis). Some cells are very motile and therefore are lost from the field of view, we record their behaviour up until the point they are lost form the field, we consider them as informative cells. The lineage data can be saved in a simple textual format for editing, analysis and re-visualisation.

Once the data is encoded, we are able to visualize the cell lineage in response to different experimental variables, such as drug dose variation. The time scaled graphical output of cell lineage comprise elaborative data about each cell at any time point. Lineage descriptors have been used to generate unique signatures of drug action. Outcome of different hypothetical situations coherent with the experimental variables can be visualized through this software which certainly facilitates the drug discovery process. Rigorous implementation of the tools described in this paper for determining the mechanism and efficacy of drug action will be presented for the exemplar anticancer agent Topotecan. We have developed tools which permit multi-dimensional data input to encode the pharmacodynam ic (PD) effects for in vitro tumour populations. Critical to realizing this aim is the drive to develop novel bioinformatics tools for microscopy data, similar to that of microarray DNA data. This requires the development and implementation of tools that enable efficient access and cross linking of multi-scalar information. Additionally this approach requires new algorithms and statistics with which to assess lineage metrics and cell behaviour relationship. Our overall strategy is to derive parametised cellular lineage meta data to inform mathematical models capable of predicting in silico cell response fingerprints for use in drug screening and herapeutics.