Context dependent changes of signalling states at single cell resolution

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Cell signalling networks can sense changes in environmental or internal state conditions, make decisions and alter effector proteins in order to trigger required responses. They can control almost every cellular process such as growth, movement, shape change and metabolism, and are often deregulated in disease. In cancer, copy number alterations and mutations often specifically target signalling genes and the consequent changes in these underlie cancer development. Decades of research into human signalling has vastly increased our knowledge of how information is integrated and decisions are made via complex relationships that include feedback regulation and pathway cross-talk. However, it is now clear that genomic alterations, tissue of origin or life history of cells can change the signaling outcomes in ways that are not well understood. A cell's context can be defined broadly as the genetic changes and other factors that stably alter its state. Context induced changes in cell states have a clear therapeutic relevance as they also alter the cell's vulnerability to perturbations such as drugs. This has been recently highlighted by the striking gene essentiality differences observed in large scale screens of cell viability upon drug exposure[1] and CRISPR screens across different cell lines[2,3]. In this project we aim to use single cell microscopy read-outs of signalling components to study how genomic alterations re-wire signalling networks and their relationship with cell morphology and motility.

Reference panels of patient tumour samples and cancer cell lines representative of different tumour types have now been characterized across a large number of genomic and molecular features. These have been extensively analysed for changes in copy number, coding sequences, gene expression, and methylation status, among others[4]. Protein and phosphoprotein abundance measurements have also been collected across several cancer types for a limited number of proteins using antibodies[5,6], and more extensively assayed for a smaller number of samples using mass-spectrometry (MS)[7,8]. These cell population based analyses have revealed very substantial changes in cell signalling associated with genomic alterations and tissue of origin. Re-analysis of these data, under-way in the Beltrao group, have identified genes whose copy number alterations or mutations are strongly associated with steady-state changes in cell signalling apparent from the proteomics data. However very little is known about how these genomic alterations change signalling networks nor how re-wired networks differ in how they process information. Importantly, most measurements to date have been collected in bulk, obscuring any underlying cell-to-cell variability, and precluding the use of pooled selection-based screens. Additionally, cell parameters such as shape and motility are not readily available for these reference panels although they are known to be important for cancer development.

In order to address these questions the ESPOD candidate will use a high throughput imaging set-up, developed in the Parts group, to follow the dynamics of signalling responses of cell lines in different natural and engineered contexts. They will quantify cell shape and motility, as well as fluorescent reporter based kinase activity from micrographs of live cells using machine learning methods established in the group, and will further develop new analysis approaches as required. The contexts will range from different genetic backgrounds in a compendium of well-characterized cancer cell lines and their derivatives with CRISPR/Cas9-induced edits, to the presence and absence of different stimulations and kinase inhibitors. Using the measured readouts, the candidate will reverse engineer the changes in signalling networks in each cell line. The changes in pathway parameters predicted by the reverse engineering approach will then be compared with the predicted changes due to the observed genetic differences. These data will also allow the candidate to

study cell-to-cell variability in the responses and its genomic determinants. This combined approach will highlight the variants most likely responsible for differences in pathway activities, cell-to-cell variability and cell morphology parameters. We expect the produced results will be highly relevant in the rationalization of context dependent cell essentiality and drug responses.

As a starting point the candidate will focus on the EGFR signalling pathway. Members of this pathway are commonly mutated in cancer, it can be synchronously activated by an external stimulus, and multiple fluorescent reporters exist to monitor its activity[9,10]. Through re-analysis of bulk population measurements, the Beltrao group will identify a panel of 30-50 cell lines where EGFR pathway genes carry genetic alterations that are representative of commonly observed changes and where EGFR signalling is changed as measured by bulk proteomics data. The applicant will implement and test known FRET and nucleocytoplasmic shuttling based reporters of kinase activities[9,10] and use these to profile the dynamics of EGF signalling across cell lines, as well as assess cell-to-cell variability in the responses. As a baseline, we will obtain measurements of cell shape and motility, known to play an important role in cell fate choice. We expect that a large proportion of the cell lines will have altered dynamical profiles of kinase activities and a subset of of these will be selected for additional experiments. In addition we will learn about the interrelationship between genomic changes, single-cell responses, cell morphology and motility.

The response to EGF will be then measured for a subset of cell lines in presence of different kinase inhibitors targeting members of the pathway allowing for the reverse engineering of the pathway changes in each cell line[11]. Finally, variant effects for all genomic changes in the EGFR pathway member genes will be predicted using mutfunc (www.mutfunc.com) and compared with the with the inferred pathway model differences. We expect that the combined approach of molecular and pathway modelling will allow us to identify the causal variants that have an impact on cell signalling in these cell lines.

In summary, we expect to gain causal understanding of how genetic variation that is recurrent in cancer re-wires cell signalling pathways, its dynamics at single cell resolution and ultimately how these changes may confer specific vulnerabilities to drug treatment.

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