Introduction

Galapagos’s mission is to develop first-in-class medicines based on the discovery of novel targets. Using human primary healthy and patient cells, the company discovers which proteins play a key role in diseases such as rheumatoid arthritis, inflammatory bowel disease, and fibrosis. One of these diseases, systemic sclerosis (SSc), also known as scleroderma, is an autoimmune connective tissue disease characterized by early vascular abnormalities and subsequent fibroblast activation which leads to fibrosis (1). Fibroblast-to-myofibroblast transition (FMT), a biological process in which fibroblasts differentiate into myofibroblasts and secrete excessive quantities of extracellular matrix (ECM) proteins, has been reported to play an important role in late-stage SSc.

Our discovery research has relied for years on high-content imaging with GE’s IN Cell Analyzer 6000 platform and the accompanying Investigator analysis software. With the advent of GE’s IN Carta software, more sophisticated target measurements have become available (2). In combination with the Phenoglyphs machine learning tools, we can now classify our samples for robust hit calling (3). With these tools, we have developed a high-throughput high-content imaging assay to measure ECM proteins in patient-derived fibroblasts. We have performed a pilot screen to assess the quality of our assay in which we will screen a library of siRNAs to identify novel drug targets.

Conclusions

Image analysis has traditionally followed a biased single feature readout where cellular marker intensity or location is used as a proxy of the cellular state. This can generate good quality assays, but these ignore the phenotypic complexity of the cell. For this systemic scleroderma FMT assay, we have taken the power of IN Carta image analysis software to evaluate many features of the selected cellular markers to incorporate more of the phenotypic complexity of the cell. The IN Carta Phenoglyphs machine learning model then allowed us to harness the most relevant of these features to create a classifier-based HCS assay for our SSc FMT assay. We validated this approach against classical single feature intensity-based scoring and found that machine learning assisted cell classification leads to a more robust assay for hit calling by doubling the Z’ of the assay. The outcome of the pilot screen gives us confidence to continue to screen for novel targets that modulate ECM deposition in SSc.

Materials and results

Assay development of a SSc HCS in patient-derived primary fibroblasts

Fig 1. Screening assay development for the SSc FMT assay. Primary diseased human skin fibroblast (FIB) cells were transfected with INV (targeting Fibronectin) and SPA (targeting SPARC) siRNA, and cell control or cell buffer only. One-day post seeding, refreshed 4 d after seeding, and harvested/fixed 7 d post seeding. ICC was performed on fibronectin using an EDA/FN antibody and a DAPI (nuclear) antibody. This assay allowed us to evaluate ECM protein production in response to siRNA perturbations. Our assay screening plates layout used multiple replicates for controls throughout the plate to observe plate and positional effects.

Image analysis of the SSc FMT phenotype with IN Carta software image segmentation

Hit calling based on phenotypic classes improves the Z’ factor of assay plates. Ultimately we validated this process for our assay with the samples and negative controls (see heatmaps).

Phenotypic classification of FMT controls by IN Carta Phenoglyphs machine learning module

Fig 3. GE Healthcare’s IN Carta Phenoglyphs™ image analysis software improves high-content screening (HCS) in scleroderma.

References