Using high content image analysis to uncover connections between YAP and cell shape

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Keywords: *morphology, YAP, GTPases, GEFs, GAPs*

Abstract

Nuclear localization of YAP, a key transcription factor involved in differentiation and breast cancer progression, is regulated by cell-cell and cell-matrix adhesion, mechanotransduction, and cytoskeletal dynamics. Although Rho family small GTPases are critical mediators of these processes, their roles in coupling YAP activation to cell morphology are still unclear. GTPases are controlled by guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs). In this study, we screened an siRNA library of human GEFs, GAPs, and small GTPases using high content automated image analysis to identify genes that regulate YAP and link its activity to cell shape. This method allows us to measure hundreds of morphological, context, and cytoskeleton features together with transcription factor localization in millions of individual cells. Perturbing GTPase signaling results in often dramatic changes in cell shape, which in turn affects YAP, which means that finding and characterizing hits for YAP activation poses a technical challenge. We used multiple regression analysis to model complex relationships between YAP and shape features, then looked for gene knockdowns that altered those relationships. Thus, we can eliminate shape-dependent effects and uncover genes that act upstream of YAP. Here we identify a number of genes that have not previously been implicated in YAP signaling, including knockdowns that affect the ratio of nuclear to cytoplasmic YAP and total levels of YAP protein. We have also identified a novel Rac GEF that appears to directly couple YAP localization to cell morphology. These findings help to elucidate how cell shape and context modulate gene expression, and highlight the importance of taking morphological factors into account when interpreting screen data.