KINOME WIDE SCREENING FOR REGULATORS OF FOCAL ADHESIONS DYNAMICS IDENTIFIES PFKFB2 AS A NOVEL DETERMINANT OF BREAST CANCER PROGRESSION

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Introduction: An essential step in metastasis formation includes tumor cell migration and invasion. This requires the plasticity of matrix adhesions structures. The migration of tumor cells is indeed highly controlled by the assembly/disassembly of those adhesions. These consist of cytoskeletal structural components, adaptor proteins, tyrosine kinases and phosphatases that together form the so-called integrin adhesome. Although some adhesome components are known to be essential in metastasis formation, it remains unclear how exactly the adhesion-mediated signaling controls the diversity of migratory and invasive behaviours of tumor cells.

Methods: To provide a systematic analysis of genes that regulate matrix adhesion dynamics, we performed a high content screen with MCF7 breast epithelial cells, using siRNAs targeting human genes encoding phosphatases and kinases. We did setup an image-based fixed assay that allows the quantification of the assembly and disassembly of the matrix adhesions in MCF7 cells using confocal microscopy. We applied the nocodazole assay described earlier by Ezratty and coworkers (Ezratty et al.,2005). Addition of nocodazole resulted in adhesion assembly while its washout provoked adhesion disassembly (Le Devedec et al., 2012). Under these conditions we fixed and stained the knockdown cells for vinculin a marker of matrix adhesions.

Results: The primary screen involved the identification of hits that impair focal adhesion assembly and/or disassembly. A validation of the hits yielded high confidence genes; some were further studied using time lapse microscopy of adhesion dynamics and tumor cell migration in different cell-lines. Importantly, one of the validated candidate genes PFKFB2, which is involved in the regulation of cell metabolism, correlated with breast cancer patient metastasis free survival.

Conclusions: Our results indicate the feasibility of automated high content imaging-based screening to identify novel clinically relevant cancer metastasis associated genes.

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