AN IMAGING-BASED TUMOR CELL MIGRATION PHENOTYPIC RNAI-SCREEN TO IDENTIFY NOVEL CANDIDATE METASTASIS GENES

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Metastasis is the major cause of death in cancer patients. Migration of cancer cells from the primary tumour to the distant site is the prerequisite of metastasis formation. Even though cancer cell migration is studied intensively, there are only few drugs available and/or in development to fight metastasis formation. We have applied parallel approaches to identify novel candidate genes involved in cancer metastasis. Firstly, we have defined the migratory and invasive behavior of large collection of human breast cancer cell lines using automated high throughput microscopy and quantitative image analysis tools. This quantitative migration/invasion phenotypic dataset was integrated with transcriptomics data of the same cell lines. A 440-gene signature was determined that was strongly correlated with both migratory and invasive phenotypes; this geneset was enriched for programs involved in cell movement and invasion. Since a cell migration phenotype-genotype correlation was strongest associated with with clinical outcome, as a second approach we used the two most motile breast cancer cell lines in RNAi screening to identify novel candidate metastasis genes. A PhagoKinetic Track (PKT) assay was used for a multi-parametric quantitative assessment of different migratory phenotypes. The image analysis was optimized to use multiple parameters to recognise migratory phenotypes (e.g. enhanced or decreased migration, loss of polarity). These phenotypes were used to identify novel candidate genes that regulate cell migration in breast cancer cells. We focused our screening effort on all cell signalling components covering all kinases, phosphatases, (de)ubiquitinases, transcription factors and G-protein coupled receptors as well as adhesion-related targets in total covering ~4,500 target genes. Candidate hits were validated in both a secondary PKT screen as well as in a tertiary live cell migration screens. Validated hits were correlated with patient breast cancer metastasis free survival. One of the candidate genes that was associated with poor patient survival, SRPK1, was successfully followed up in an *in vivo* mouse model for breast cancer metastasis. Our current systems microscopy approach is a powerful tool to identify novel regulators of cancer cell migration that are of clinical relevance to breast cancer metastasis and may ultimately serve as candidate drug targets to combat cancer progression.

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