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Analysis of a metastasising sarcoma by quantitative light microscopy provides evidence of a role for protein 4.1B

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A model of metastasis was established by the tumour progression of spontaneously transformed embryonic fibroblasts derived from inbred Lewis rats. This process resulted in a collection of sarcoma cell lines with different abilities to shed metastases in the inbred rats. Cell behaviour in vitro was analysed using quantitative light microscopy and we found that the metastatic phenotype was accompanied by changes in cell morphology including a loss of actin stress fibres and significantly increased speed of cell motility. In order to reveal the underlying patterns of changes in gene expression, we performed microarray analysis using both tissue culture cells and primary tumours. The Affymetrix microarrays we used contained probes for about 11,000 genes and statistical analysis showed that there were 23 genes with significantly changed expression levels of more than 2.5-fold in both tissue culture cells and primary tumours. The changes in expression were confirmed for 10 selected genes using RTPCR. Two of these genes, Bk and Ril, were strongly upregulated in the metastasising cells but their over-expression in experiments with the non-metastasising cells did not produce any obvious phenotypic changes. Similarly a 32-fold downregulated gene Cask produced no evident phenotypic changes when over-expressed in the metastasising cells. Protein 4.1B was downregulated 36-fold in metastasising cells, and RNAi of 4.1B in the non-metastasising cells resulted in acquisition of significant features of the metastasising cells, namely loss of actin stress fibres and enhanced motility. We conclude that 4.1B protein plays a role in metastasis by promoting a motile phenotype.