Epigenetic drivers of melanoma metastasis identified by genome-wide reduced-representation bisulphite sequencing (RRBS) analysis

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As cancer metastasis is a major cause of cancer-related mortality, understanding the causes and effects of genomic changes in this process will help in identifying metastasis-related treatment targets. We have focused on investigating genomic changes in melanoma metastasis, because melanoma is one of the most aggressive and invasive of human cancers. While epigenetic alterations are hypothesized to play a facilitatory role in metastasis, for example in the promotion of epithelial to mesenchyme transition (EMT), which is increasingly supported by accumulating evidence, it is not clear whether epigenetic changes are necessary or sufficient to *drive* metastasis. We have addressed this question by using an approach involving RRBS mapping of genome-wide DNA methylation changes in order to identify shared methylation changes across a series of primary and metastatic matched melanoma samples. Using this approach we identified 75 common (10 hyper- and 65 hypomethylated) genomic regions associated with 68 genes showing significant methylation differences between metastatic and primary tumours, which were shared amongst all three unrelated metastatic melanoma cell lines. One epigenetic change involved elevated *Early B Cell Factor 3* (*EBF3*) mRNA levels and concomitant promoter hypermethylation in all three metastatic melanoma cell lines. RNAi-mediated knockdown of *EBF3* in melanoma cell lines demonstrated an oncogenic role for *EBF3* expression in promoting aggressive melanoma behaviour. We investigated collections of additional melanomas, and identified similar significant promoter hypermethylation and significantly elevated *EBF3* mRNA levels in metastatic versus primary melanomas in two publicly available independent melanoma cohorts (n=40 and 458 melanomas, respectively). Moreover *EBF3* has recently been identified as a top hit in GWAS screening of melanoma susceptibility loci. Including ours, several studies now suggest that epigenetic alterations have the potential to be oncogenic "drivers" of cancer aggressiveness, and are commonly recruited during the process of metastatic progression in tumour cells.