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MicroRNA-1246 Expression Associated with Resistance to Oncogenic BRAF Inhibitors in Mutant BRAF Melanoma Cells

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Abstract : Intrinsic and acquired resistance limits the therapeutic benefits of oncogenic BRAF inhibitors in melanoma. MicroRNAs (miRNA) regulate the expression of target mRNAs by repressing their translation. Thus, we investigated miRNA expression patterns in melanoma cell lines to identify candidate biomarkers for acquired resistance to BRAF inhibitor. Here, we used Affymetrix miRNA V3.0 microarray profiling platform to compare miRNA expression levels in three cell lines containing BRAF inhibitor-sensitive A375P BRAF V600E cells, their BRAF inhibitor-resistant counterparts (A375P/Mdr), and SK-MEL-2 BRAF-WT cells with intrinsic resistance to BRAF inhibitor. The miRNAs with at least a two-fold change in expression between BRAF inhibitor-sensitive and –resistant cell lines, were identified as differentially expressed. Averaged intensity measurements identified 138 and 217 miRNAs that were differentially expressed by 2 fold or more between: 1) A375P and A375P/Mdr; 2) A375P and SK-MEL-2, respectively. The hierarchical clustering revealed differences in miRNA expression profiles between BRAF inhibitor-sensitive and –resistant cell lines for miRNAs involved in intrinsic and acquired resistance to BRAF inhibitor. In particular, 43 miRNAs were identified whose expression was consistently altered in two BRAF inhibitor-resistant cell lines, regardless of intrinsic and acquired resistance. Twenty five miRNAs were consistently upregulated and 18 downregulated more than 2-fold. Although some discrepancies were detected when miRNA microarray data were compared with qPCR-measured expression levels, qRT-PCR for five miRNAs (miR-3617, miR-92a1, miR-1246, miR-1936-3p, and miR-17-3p) results showed excellent agreement with microarray experiments. To further investigate cellular functions of miRNAs, we examined effects on cell proliferation. Synthetic oligonucleotide miRNA mimics were transfected into three cell lines, and proliferation was quantified using a colorimetric assay. Of the 5 miRNAs tested, only miR-1246 altered cell proliferation of A375P/Mdr cells. The transfection of miR-1246 mimic strongly conferred PLX-4720 resistance to A375P/Mdr cells, implying that miR-1246 upregulation confers acquired resistance to BRAF inhibition. We also found that PLX-4720 caused much greater G2/M arrest in A375P/Mdr cells transfected with miR-1246mimic than that seen in scrambled RNA-transfected cells. Additionally, miR-1246 mimic partially caused a resistance to autophagy induction by PLX-4720. These results indicate that autophagy does play an essential death-promoting role inPLX-4720-induced cell death. Taken together, these results suggest that miRNA expression profiling in melanoma cells can provide valuable information for a network of BRAF inhibitor resistance-associated miRNAs.

Keywords : microRNA, BRAF inhibitor, drug resistance, autophagy
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