miRNAs in the 14q32 cluster are involved in lapatinib resistance

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HER2 is over-expressed in around 20-25% of breast cancers. Due to increased cell growth and differentiation signaling resulting from HER2 over-expression, this sub-type of the disease is associated with aggressive tumor progression and poor prognosis. HER2 targeted therapy has improved patient prognosis, however, only a subgroup of patients derives the optimal benefit, while other patients have refractory disease or develop resistance. miRNAs are small non coding RNAs involved in the RNAi machinery. They are often aberrantly expressed in tumors and miRNA profiles have been seen to evolve over the course of treatment implicating them in therapeutic resistance.

Methods

miRNA array completed on the Human TaqMan miRNA card. Gene expression profiling performed by Illumina Human HT12 v4.0 array. Differential gene expression used LIMMA1 empirical Bayes statistics module. Adjusted p-values calculated by Benjamini and Hochberg procedure. In silico target prediction used miRWalk. Lapatinib resistant cells generated by culturing cells in increasing doses of lapatinib up to 2μM. IC50 obtained from drug cytotoxicity measured by MTT analyzed by nonlinear regression.

Results

A panel of miRNAs differentially expressed in paired lapatinib sensitive/resistant BT-474 HER2 positive breast cancer cells were identified by miRNA array. Three miRNAs, located in the 14q32 region, were selected for further investigation of their role in resistance to HER2-targeted therapy: miR-127-3p, miR-409-3p, and miR-495-3p. RT-qPCR also confirmed upregulation of these miRNAs in HCC1954 and SKBR-3 HER2 positive cells with acquired resistance to lapatinib, linking increased miRNA expression with acquired resistance. To understand the mechanism behind the miRNA expression in resistant cells we explored epigenetic changes between the sensitive and resistant lines. Global methylation reversal revealed upregulation of all miRNAs in the sensitive cells with a further increase upon addition of an HDAC inhibitor. De-methylation had less effect in resistant cells, indicating loss of methylation as a key component in miRNA upregulation in the process of lapatinib resistance. Using differential gene expression analysis in paired lapatinib-sensitive/resistant BT-474 cells combined with in silico analysis we identified putative targets of these miRNAs. BASP1, a c-MYC transcriptional inhibitor, was down-regulated in the BT-474/L resistant cells. Knock-down altered sensitivity to lapatinib, with an 8 fold shift in IC50 towards resistance, indicating reduced expression of the protein could be involved in the resistance machinery.

Conclusions

miR-127, miR-409, and miR-495, clustered to the 14q32 region are subject to epigenetic control, which is altered in lapatinib resistant cells. Resulting upregulation of the miRNAs appears to post-transcriptionally regulate genes, such as BASP1, involved in an acquired resistance mechanism to lapatinib in HER2 positive breast cancer cell lines.