Differentially expressed microRNAs and the antiproliferative role of miR-126 in small cell lung cancer

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Summary

MicroRNAs are small, non-coding RNAs that regulate gene expression by binding to the 3' untranslated region (3'UTR) of target mRNAs, inducing mRNA degradation or translation repression. Approximately 50% of miRNA genes are in cancer-associated genomic regions, suggesting that microRNAs play a significant role in tumor biology. Small cell lung cancer (SCLC) is a high-grade neuroendocrine tumor characterized by rapid progression and frequent metastasis.

We combined microarray and qRT-PCR analyses to identify microRNAs aberrantly expressed in small cell lung cancer. The microarray approach alone identified 19 miRNAs that are significantly overexpressed, by at least 10-fold in SCLC cell lines compared to normal lung. We also identified 35 miRNAs that are downregulated in SCLC cell lines compared to normal lung, and a large region in 19q13.41 containing more than 30 downregulated miRNAs (miR-512 to miR-373). RNA samples from SCLC cell lines and a small number of microdissected primary SCLC tumors were analyzed with qRT-PCR as well. At first we identified 16 overexpressed and 8 downregulated miRNAs in primary SCLC tumor samples, as well as in SCLC cell line samples. 7 downregulated and 8 overexpressed miRNAs were selected for further analysis in a larger panel of individual SCLC tumors and SCLC cell lines. qRT-PCR analysis verified that miR-126 is a uniformly downregulated miRNA, while miR-301 and miR-183 are uniformly overexpressed microRNAs in all SCLC sample types. We analyzed DNA copy number changes in primary SCLC tumors for 5 genomic regions with overexpressed miRNAs. We identified one novel amplified region in SCLC: 7q32.2 contains the miR-183/96/182 cluster.

In our further work we demonstrated that miR-126 overexpression has a negative effect on SCLC cell proliferation, by delaying cells in the G1 phase of the cell cycle. Importantly, we identified SLC7A5 as a novel target of mir-126 in SCLC cells. miR-126 downregulates the expression of SLC7A5 at the translation level, and reduces mRNA stability simultaneously. We demonstrated that in SCLC cells, similarly to other tumor types, suppression of SLC7A5 expression has an anti-proliferative effect. SCL7A5 suppression or miR-126 overexpression both delay SCLC cells in the G1 phase, suggesting that the effect of mir-126 on the cell cycle is mediated at least in part through SLC7A5. SLC7A5 provides the essential amino acids that act as signal to enhance growth of cancer cells through mammalian target-of-rapamycin (mTOR)-stimulated translation. Through different targets, miR-126 can

negatively regulate PI3K/Akt pathway, which is aberrantly active in a large percentage of SCLC tumors. Therefore, miR-126 is an important negative regulator of the growth and proliferation of SCLC cells, which probably fine-tunes the activity of the PI3K/Akt/mTOR network through multiple targets, including SLC7A5.

KEYWORDS

microRNA, small cell lung cancer, miR-126, SLC7A5, G1 delay

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