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For these studies, we chose to use miR-675-5p as a positive control and GPR55 as the target gene. This article focuses on the differences in gene expression in THP-1 cells, compared to GPR55, which we observed with GPR55 mRNA levels. Most of the genetic alterations include gene mutations and copy number variations (CNVs). However, recent studies have suggested that miRNAs can regulate gene expression by directly interacting with target mRNAs. Large-scale miRNAs profiling can provide valuable information about the molecular pathogenesis and clinical outcome of lung cancer [ 15 - 17 ]. We chose to investigate whether GPR55 is a target of miR-675-5p, and to explore the possibility that it can be a target of cancer-related miRNAs in NSCLC. To this end, we used data from The Cancer Genome Atlas (TCGA) lung adenocarcinoma miRNAs profiles and the results showed that the GPR55 expression level was significantly higher than that of the THP-1 cells compared to the gene expression profile. This result demonstrates that GPR55 is significantly up-regulated in lung adenocarcinomas compared to the THP-1 cells. And, the predicted target miRNA by microRNA.org was miR-675-5p and the 3'-untranslated region (3' UTR) of GPR55 was the predicted binding sites on the predicted target site by microRNA.org. The expression level of GPR55 mRNA with over-expression of miR-675-5p was significantly decreased in THP-1 cells when compared to the control. Compared to the empty vector, the 3'-UTR luciferase activity was decreased. These results demonstrate that miR-675-5p is able to post-transcriptionally regulate GPR55 expression. The study also provides a proof-of-principle example that miRNAs have great potential in cancer therapeutics. Over-expression of miR-675-5p could efficiently inhibit cell proliferation via GPR55 down-regulation. And, it may be a potent miRNA therapeutic in the treatment of lung cancer.

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Recently, powerful high-throughput RNA sequencing technology has led to a widespread interest in examining the role of miRNAs in cancer. miRNAs may be attractive biomarkers for various cancers, either as novel diagnostic and/or prognostic markers [ 22 - 24 ]. As a result, expression of miRNAs in lung cancer tissues may be useful for diagnosis and prognosis of the malignancy. Here, we have determined the expression of miR-675-5p in NSCLC cell lines and tumor tissues, and shown that miR-675-5p is down-regulated in NSCLC tissues compared with paired normal tissues. Overexpression of miR-675-5p in NSCLC cells suppressed tumor cell proliferation, invasion and migration and promoted apoptosis and autophagy. In addition, we identified several direct targets of miR-675-5p, including 3-phosphoinositide-dependent protein kinase 1 (PDK1), protein-tyrosine phosphatase, non-receptor type 22 (PTPN22), enhancer of mRNA-decapping protein 4 (EDC4) and E3 ubiquitin protein ligase TRIP12. Finally, we demonstrated that knockdown of PDK1 was able to partially reverse the tumor-suppressive effects of miR-675-5p. Therefore, we hypothesized that miR-675-5p is a novel and effective tumor suppressor in NSCLC. Klotho (KL) protein, the gene encoding the Klotho protein, is a single-pass transmembrane protein consisting of 695 amino acids. The extracellular domain of KL protein is highly conserved, while its primary sequences are significantly different from those of other similar proteins. Extracellular KL protein has only one gene transcript, and the mRNA is composed of exons 1, 2, and 3. The KL-1 transcript is 825 bp and contains exon 1, exon 2, and exon 3, while the KL-2 transcript is 1275 bp and contains exon 1, exon 2, and exon 3 (Fig1B). KL-1 protein is 400 amino acids, and the KL-1 protein contains a KL domain [9]. Klotho also contains a homologous domain of 135 amino acids with the C-terminal domain of transthyretin (TTR) protein [10]. KL protein is predominantly expressed in kidney, the lung, and the vascular system [11-13], and KL-1 protein is mainly expressed in the distal nephron [14]. KL-1 deficiency or mutations in KL-1 protein lead to multiple aging-related syndromes (MAS), including aging-associated Parkinsonism, type 2 diabetes mellitus, cataracts, and cardiovascular disease [15, 16]. 5ec8ef588b

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