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Small molecule targeting miR-34a for cancer therapy

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miR-34a functions as a tumor suppressor in many cancers. In a recent issue of Cancer Research, we describe a small molecule, Rubone, with strong anticancer activity. Rubone selectively restored miR-34a expression in hepatocellular carcinoma cells in which miR-34a was silenced. Restoration of miR-34a by Rubone represents a promising therapeutic approach against cancer.

MicroRNAs (miRNAs) are a class of short noncoding RNAs that negatively regulate gene expression. More than 2,500 mature miRNAs have been discovered in the human genome and approximately 25%–70% of human genes are regulated by miRNAs.[1] In cancer cells, miRNAs might function as oncogenes or tumor suppressors and their roles are manifested in almost all aspects of cancer biology including proliferation, apoptosis, invasion/metastasis, and angiogenesis.[2] Therefore, miRNAs are ideal targets for anticancer drug development. MicroRNA-34a (miR-34a) functions as a tumor suppressor and is downregulated or silenced in a variety of human cancers. Thus, restoration of miR-34a might represent a potential therapeutic approach for such cancers. Small molecules are low molecular weight compounds that have a potent biological effect. Many commonly used anticancer drugs, including imatinib, gefitinib, erlotinib, and sorafeinb, are small molecules. Small molecules provide significant advantages for anticancer drug development: a wide range of compounds can be synthesized in a relatively short time; the compounds can be easily screened for specificity and capacity of binding with a target; and most importantly, small molecules are orally bioavailable and their manufacturing is cost-effective,[3] which is an important advantage for patients and healthcare systems. These advantages have led researchers to pursue compounds targeting miR-34a for anticancer drug development.

Over the last several years, researchers have been investigating small molecules as miRNA modulators. Some miRNA modulators with potential biological activities were identified, such as miR-21 and miR-122 modulators;[4,5] however, the screening of miRNA modulators is far from sufficient. In the study described in the current issue of Cancer Research[6] we used a hepatocellular carcinoma (HCC) cell-based miR-34a luciferase reporter system to screen for miR-34a modulators that could exert anticancer activity, as shown in **Figure 1**. One small molecule compound called Rubone was identified as a miR-34a modulator in HCC cells. Rubone activated miR-34a expression in HCC cells with wild type or mutated TP53 (TP53, best known as p53), but not in cells with p53 deletions. Notably, Rubone lacked growth inhibitory effects on non-tumorigenic human hepatocytes. In a mouse xenograft model of HCC, Rubone dramatically inhibited tumor growth, exhibiting stronger anti-HCC activity than sorafenib both *in vitro* and *in vivo*. Mechanistic investigations showed that Rubone decreased expression of CYCLIN D1, BCL-2 (B-cell lymphoma 2), CDK6 (cyclin-dependent kinase 6), SIRT1 (Sirtuin 1), and FOXP1 (Forkhead box protein P1) and enhanced the occupancy of p53 on the miR-34a promoter. Taken together, our findings provide a preclinical proof of concept for Rubone as a lead

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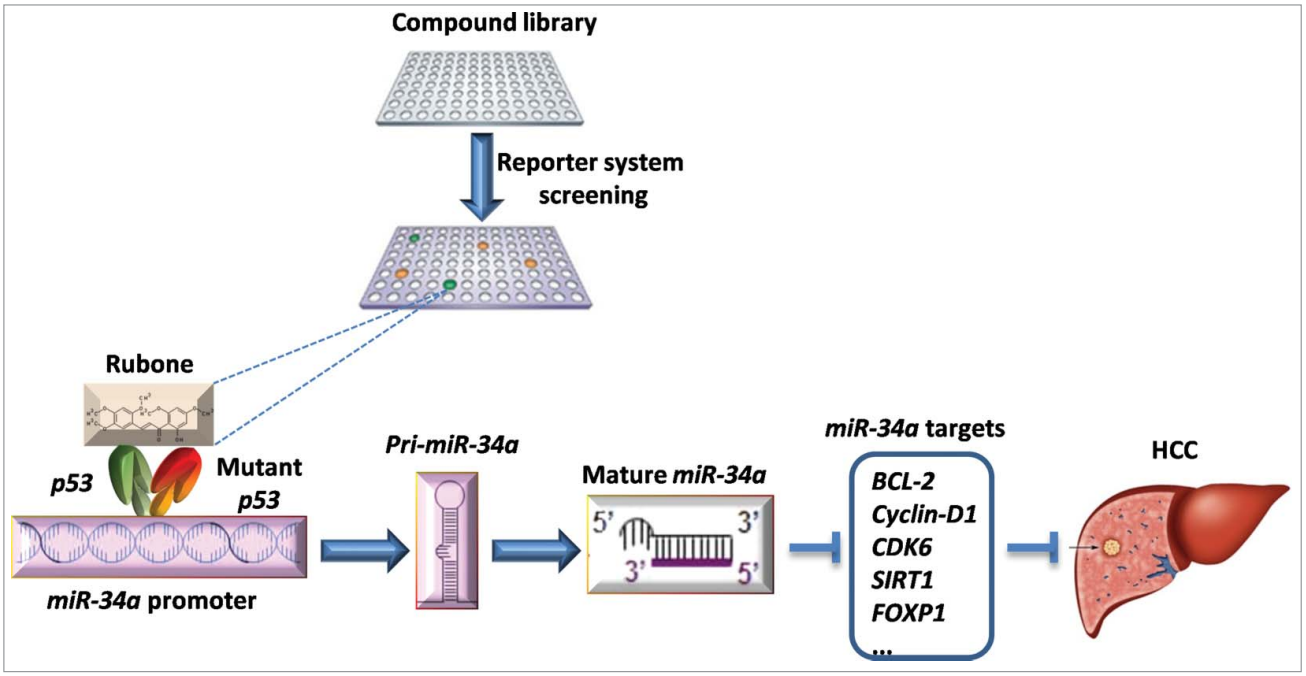

Figure 1. Screening of p53-dependent miR-34a modulators that inhibit hepatocellular carcinoma. Schematic representation of screening for miR-34a modulators and the identification of one compound, called Rubone, that increased both wild-type and mutant p53 occupancy on the miR-34a promoter. Rubone increased miR-34a expression and subsequently downregulated the expression of miR-34a targets such as CYCLIN D1, BCL-2 (B-cell lymphoma 2), CDK6 (cyclin-dependent kinase 6), SIRT1 (Sirtuin 1), and FOXP1 (Forkhead box protein P1), ultimately inhibiting HCC growth.

candidate for further investigation of a new class of HCC therapeutics based on restoration of miR-34a tumor suppressor function.

The major strengths of this study include screening for a specific miR-34a modulator with strong anti-HCC activity and elucidation of the detailed mechanism underlying modulation of miR-34a expression by Rubone. With the recent entry of miRNA-directed therapeutics into Phase II clinical trials,[7] it is a propitious time to apply our findings to the future development of novel anticancer therapies. Another novel discovery from this study is the demonstration that mutant p53 is also involved in the tumor suppressive function of miR-34a. Although previous studies showed that p53 directly regulated the expression of miR-34a and more than 50% of human tumors contain mutation or deletion of p53 gene,[8] there is no report on the relationship between miR-34a and mutant p53. Our study may provide a new clue to the multifunctional roles of mutant p53 in cancer. This study also gives direction for the development of a new class of anticancer compounds targeting mutant p53, through which the tumor suppressive function of mutant p53 could be recovered.

Despite the strengths of this paper, several potential issues arising from our studies need to be addressed in the future. First, we found that Rubone specifically inhibited the growth of HCC cells with wild-type and mutated p53; however, it seems that the tumor suppressive function of this compound is limited in p53-deleted cells. Since p53 is deleted in some cancer cells, novel miR-34a modulators that could function in p53-deleted cells should be screened. Second, miR-34a also plays an important role in some other diseases, such as metabolic diseases and cardiac diseases. Although it is an advantage that Rubone lacked miR-34a modulator effects on non-tumorigenic human hepatocytes, this also indicates that Rubone may not work in metabolic or cardiac diseases. Thorough assessment of the effects of Rubone on these diseases is required. Furthermore, Rubone may be chemically modified for miR-34a modulator function in the abovementioned diseases. Third, the mechanism of action of Rubone as a miR-34a modulator involves significantly increased p53 occupancy on the miR-34a promoter in HCC cells. However, the effect of Rubone on p53 is still unknown. Rubone may restore or enhance a particular molecular function of p53, either in a normal state for wild-type p53 or a suboptimal state for mutant p53. For example, Rubone may enhance the DNA-binding function of p53 protein on miR-34a promoter by modulating the C-terminal domain of p53 protein or enhance the ability of p53 to recruit co-factors such as GADD45A (growth arrest and DNA damage-inducible, α), PCNA (proliferating cell nuclear antigen) and APE1 (human AP endonuclease)[9,10] These issues require further investigation.

Overall, this study showed that the modulatory effects of a small molecule on a small RNA can have large effects in cancer. This is not only the first report of a miR-34a modulator with

obvious anti-HCC activity but also provides a clue to the development of miR-34a modulators dependent on wild-type or mutant p53, which represents a bright prospect for developing further modulators of miRNAs for disease treatment.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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