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Downregulations of B-cell lymphoma 2 and myeloid cell leukemia sequence 1 by microRNA 153 induce apoptosis in a glioblastoma cell line DBTRG-05MG

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MicroRNA-153 (miR-153) is a brain-specific miRNA that is expressed at a significantly lower level in glioblastoma (GBM) relative to non-neoplastic brain tissue. Although the expression pattern of miR-153 has been extensively established, its target genes and cellular mechanism remain undefined. To investigate into the potential function of miR-153 in glioblastmas, we transfected a GBM cell line DBTRG-05MG with synthetic miR-153 oligos and observed decreased cell proliferation and increased apoptosis. Bioinformatics analysis revealed that anti-apoptosis family member B-cell lymphoma 2 (Bcl-2) and myeloid cell leukemia sequence 1 (Mcl-1) are potential targets of miR-153. Indeed, Western blot analysis indicated that miR-153 downregulated both Bcl-2 and Mcl-1 at the protein levels. Single strand miR-153 inhibitor, which forms complementary base-pair with endogenous miR-153, efficiently blocked the apoptosis and target protein degradation induced by overexpression of miR-153. By luciferase reporter assays, we further showed that miR-153 inhibited Bcl-2 and Mcl-1 expressions by directly targeting the 3′UTR regions of their respective mRNAs.

MicroRNAs (miRNAs) are a group of ~22-nucleotide long endogenous noncoding RNAs that can repress protein translation or cleave mRNAs through binding to target sites located at the 3′UTR regions of target mRNAs.[1] MiRNA-directed gene silencing is mediated *via* RNA-induced silencing complex (RISC), a multi-subunit RNA binding protein complex with nucleases activity.[2] Previous studies suggested that 5′-ends of miRNAs are crucial for recognition of target sites and proper biological function.[3,4] The first 2–8 bases of a particular mature miRNA sequence, referred to as the "seed" region, is commonly used in most bioinformatics algorithms to search for complementarities to sequences in the 3′UTR regions of potential target mRNAs.[5] The fundamental regulatory roles of miRNAs have been linked to diverse cellular processes including malignant proliferation, apoptosis and differentiation. Inappropriate regulations of miRNAs are also associated with several types of diseases such as cancers and viral infections.[1,6]

Glioblastoma (GBM) is the most aggressive manifestation of malignant gliomas and considered to be among the deadliest form of human cancers.[7] Because miRNAs are found to tightly regulate diverse biological processes and considered to play important roles in cancer etiology, it is reasonable to hypothesize that certain important miRNA regulators would be associated with GBM and implicated in tumor formation. Several groups have set up large scale miRNAs expression analysis to characterize miRNA expression profiles in central nervous system (CNS) tumor-derived cell lines or clinical brain tumor samples.[8,9] The expressions of a few miRNAs have indeed been shown to be significantly changed in GBM samples relative to non-neoplastic brain tissues. miR-153 was first discovered as 1 of the 7 brain-specific miRNAs (miR-9, -124a, -124b, -135, -153, -183 and -219) based on expression analysis of 119 miRNAs in adult organs from mouse and human using Northern blot analysis.[10] Because the expression levels of miR-153 in CNS tumor-derived cell lines are about 188-fold less when compared with the normal tissue, miR-153 has been proposed as a potential tumor suppressor.[8] Recently, Silber *et al.* [9] used quantitative RT-PCR to assess miRNA expression in high-grade human astrocytomas. They reported that the expression level of miR-153 was also significantly decreased in GBM multiforme (World Health Organization grade IV) relative to non-neoplastic brain tissue. Although the expression pattern of miR-153 has been

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