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Identification of miRNA-OGG1 mRNA interactions: small RNA sequencing and immunoprecipitation analysis

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Reactive oxygen species induce modifications of the DNA bases that are implicated in cancer development and progression as well as aging and age-related neurological disorders. The base excision repair mechanism had evolved to repair the mutations induced by oxygen radicals. The objective of this study is to identify novel microRNAs that regulate the expression of 8-oxoguanine glycosylase (OGG1), an enzyme that plays an important role in the DNA base excision repair pathway. Altered expression of OGG1 leads to accumulation of modified bases, DNA damage, and increased rate of nucleic acid mutation. To simulate conditions of oxidative stress, human astrocytes were treated for 16 hours with 10 μ M sodium dichromate. OGG1 mRNA and protein expression levels were assessed via RT-qPCR and protein simple Wes® assay. RNA extracted from treated and non-treated cells was sequenced using Ion Proton small RNA sequencing platform. OGG1 mRNA and protein expression levels were significantly reduced after treatment with sodium dichromate. MicroRNA sequencing revealed that large numbers of microRNAs are upregulated following treatment with sodium dichromate. Bioinformatics analysis was implemented to identify potential microRNAs that bind to the 3'UTR region of the OGG1 mRNA gene, which includes miR-20b, miR-33, miR-let7, miR-103, and miR-491. The most statistically significant microRNA candidate, miR-103 was further employed in immunoprecipitation studies using the MirTrap System. Co-transfection of astrocytes with miR-103 mimic and the pMirTrap vector resulted in co-immunoprecipitation of miR-103–OGG1 complex which was validated by qRT-PCR, with an OGG1 mRNA fold enrichment of up to 7.