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IDENTIFICATION OF A MIR-212 TARGET SITE IN KIR2.1 3′ UTR BY A NOVEL DUAL-FLUORESCENCE MICRORNA TARGETING ASSAY

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MicroRNAs (miRNAs) are increasingly recognised as important down-regulators of gene expression in health and disease. Luciferase-based assays are commonly used for identifying miRNA targets but these have limitations. We report miRNA target identification using a novel functional miRNA targeting assay. The 3′ UTR of the human inward rectifier K+ channel Kir2.1 was inserted downstream of the mCherry red fluorescent protein sequence in a mammalian expression plasmid. MiRNA or non-targeting control (SCR) sequences were inserted into the pSM30 expression vector. Enhanced green fluorescent protein expression is an indicator of miRNA expression. HEK293 cells stably expressing the mCherry-Kir2.1 3′ UTR construct were transfected with the pSM30-based plasmids. The principle is that functional targeting of the 3′ UTR by the miRNA decreases the cell red/green fluorescence intensity ratio. It was validated with miR-1, a known downregula tor of Kir2.1, and used to investigate targeting of the Kir2.1 3′ UTR by miR-212, as predicted by bioinformatics. Mir-212 is upregulated in heart failure, which features downregulation of inward rectifier K+ channel activity in its pathophysiology. Red/green ratio was significantly lower in miR-1-expressing and miR-212-expressing cells versus non-targeting controls (miR-1 0.50±0.012 (mean±sem), n=776; miR-212 0.72±0.024, n=550; SCR 1.21±0.025, n=731; $p<0.001$ for miR-1 vs SCR and miR-212 vs SCR). The miR-212 effect was attenuated by mutating the predicted target site (% inhibition 58.0±14.51, n=3 wild-type; 22.7±1.25, n=3 mutant; $p<0.05$). This novel assay has several advantages over luciferase-based assays including larger sample size, amenability to time course studies, and adaptability to high-throughput screening.