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MicroRNAs Profiling Reveals a Potential Link between the SDG8 Methyltransferase and Brassinosteroid-Regulated Gene Expression in *Arabidopsis*

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MicroRNAs are a class of short non-coding RNAs (17-27 nucleotides) found in animals and plants. MicroRNAs play important roles in post-transcriptional regulation of gene expression by complementing the target mRNAs and causing translational repression or target mRNA degradation [1]. Studies have shown that thousands of human protein-coding genes are regulated by microRNAs [2], impacting many important biological processes, from development to physiology to stress response. In plants, microRNAs have been implicated in multiple essential biological processes, such as leaf morphogenesis and polarity, flower development, hormone signaling and metabolism, and stress responses [3]. MicroRNA genes are transcribed by RNA polymerases II, generating precursors that undergo a series of cleavage events to form mature microRNA [4]. MicroRNA biogenesis and its expression regulation are highly complicated [5]. Although the biological importance of microRNAs is well demonstrated in a wide range of cellular processes, how microRNA expression and abundance are regulated is not fully understood.

Histone modifications play critical roles in regulation of gene expression in eukaryotes [6]. Acetylation of histones H3 and H4 is commonly associated with gene activation, whereas histone deacetylation generally leads to gene silencing. Trimethylation of histone H3 on lysine 4 (H3K4me3) is frequently associated with active transcription, while trimethylation of histone H3 on lysine 27 (H3K27me3) is often associated with gene repression. Trimethylation of histone H3 on lysine 36 (H3K36me3) is usually enriched in coding regions of actively transcribed genes [6], but H3K36me3 also correlates with gene silencing in facultative and constitutive heterochromatin[7]. Recent studies in mammalian cells indicate that histone acetylation and methylation affect microRNA expression. Scott et al. [8] first showed that histone deacetylase inhibition results in alteration of microRNA levels in a breast cancer cell line. Following this study, several reports have shown that histone deacetylase inhibition alters microRNA expression in human carcinomas [9-11]. Overexpression of histone deacetylases in chronic lymphocytic leukemia results in silencing of miR-15a, miR-16, and miR-29b, while histone deacetylase inhibition can partially restore the expression of miR-15a, miR-16, and miR-29b [12]. These results collectively show that histone acetylation and deacetylation play important roles in regulation of microRNA expression in human cells. Stable RNAi-mediated suppression of the H3K4me3 demethylase JARID1B in breast tumor cells caused increased expression of several members of the let-7 family of microRNAs, suggesting that H3K4me3 is required for up-regulation of the expression of these microRNAs [13]. Parallel sequencing analyses show that H3K27me3 is associated with repressed microRNA genes in mouse lymphocytes [14]. Taken together, these studies indicate that histone methylation can modulate microRNA expression.

The SET domain group 8 (SDG8) protein is the primary methyltransferase for global histone H3K36 trimethylation in Arabidopsis [15,16]. SDG8 is involved in a number of developmental processes such shoot branching, ovule and anther development, and flowering time [16-18]. To explore the potential role of histone H3K36 trimethylation in the regulation of microRNA expression, we compared the miRNA expression profile of the knockout mutant of the SDG8 methyltransferase (sdg8) with the wild-type (WT) using microRNA microarray technology. MicroRNA microarray analysis was carried out out by LC sciences (Houston, Texas, USA) on µParaflo™ microfluidics chips containing 154 microRNA probes to *Arabidopsis* microRNAs (Sanger miRBase Version 9.2). Of the 154 microRNAs examined, 23 were differentially expressed in sdg8 knockout mutant. Twelve of 23 were overexpressed [log2 (sdg8/WT) range: 0.22 to 3.09], whereas 11 of 23 were underexpressed [log2 (sdg8/WT) range: -0.32 to -1.42]. All 23 differentially expressed microRNAs had a q-value <0.01, indicating that the observed changes were significantly different. Differentially expressed microRNAs in sdg8 knockout mutant with $|\log2\ (sdg8/WT)|$ values greater than 1 are listed in Table 1. These results suggest that a small number of known microRNAs examined are regulated by the SDG8 methyltransferase.

Brassinosteroids are a class of plant hormones that regulate multiple aspects of physiological responses essential to growth and development [19]. MicroRNA expression profiling of sdg8 knockout mutant leaves revealed that 5 microRNAs were up-regulated more than 2-fold compared to wild type leaves (Table 1). Two of these, miR395a and

microRNA	log2 (sdg8/WT)	Fold change
ath-miR843	3.09	8.52
ath-miR395a	2.48	5.58
ath-miR395b	2.37	5.17
ath-miR854a	1.41	2.66
ath-miR156h	1.06	2.09
ath-miR824	-1.42	-2.68
ath-miR822	-1.32	-2.50
ath-miR391	-1.01	-2.01

Positive values indicate increased microRNA expression in the sdg8-4 knockout mutant compared to wild type (WT), whereas negative values indicate decreased microRNA expression in the sdg8-4 mutant

Table 1: Differentially expressed microRNAs in sdg8 mutant leaves identified by microarray analysis.

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microRNA	Target accession	Target description	Target regulation by brassinosteroid
ath-miR156h	AT3G57920	Squamosa promoter binding protein-like 15	up
ath-miR822	AT5G02330	Cysteine/Histidine-rich C1 domain family protein	up
ath-miR824	AT3G12470	Polynucleotidyltransferase	up
ath-miR843	At3g13840	GRAS family transcription factor	up
ath-miR854a	AT5G05090	Homeodomain-like superfamily protein	up
ath-miR391	AT3G02380	CONSTANS-like 2 protein	down

MicroRNA targets were predicted using psRNAtarget program (http://plantgrn.noble.org/psRNATarget/) with default parameter settings
Brassinosteroid-responsive genes were determined by the AtGenExpress database (Arabidopsis eFP Browser, http://bbc.botany.utoronto.ca/efp/cgi-bin/efpWeb.cgi?dataSource=Hormone)

Table 2: Predicted microRNA targets that are brassinosteroid-responsive genes.

miR156h, have been shown to be up-regulated by 24-epibrassinolide (a highly active brassinosteroid) [20]. Taken together, these results suggest that the SDG8 methyltransferase plays a role in modulation of brassinosteroid-regulated microRNA gene expression.

Next we searched for potential target genes of the differentially expressed microRNAs in sdg8 knockout mutant using the psRNATarget program [21]. Brassinosteroid-responsive genes were identified from the hormone response data using Arabidopsis eFP Browser [22]. The potential microRNA target genes being brassinosteroid-responsive are listed in Table 2. The data mining analysis reported here implies that there is a link between the SDG8 methyltransferase and brassinosteroid-regulated gene expression in *Arabidopsis*, as the SDG8 homologue in rice, SDG725, has been shown to be critical in modulating brassinosteroid-related gene expression [23].

In conclusion, our microarray data indicate that the H3K36 methyltransferase SDG8 can modulate the expression of certain microRNA genes in Arabidopsis. Data mining analysis suggest a link betweenthe SDG8 methyltransferase and brassinosteroid-regulated gene expression in *Arabidopsis*.

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